Thesis: "The Parathyroid Glands and Parathyroid Surgery in End Stage Renal Failure"

Dr Joseph Do Woong Choi MBBS, BSc (Adv)

Date of submission: December, 2017

A thesis submitted for th**e** degree of Master of Philosophy

(Surgery) of The Australian National University

PART I: PREFACE

Declaration of Authorship and Research

I hereby declare that this submission is my own work, and to the best of my knowledge, it contains no materials previously published or written by another person, except where due reference has been made within the text. In addition, there are no substantial proportions of material which have been accepted for the award of any other degree or diploma at Australian National University or any other educational institution. The author acknowledges that the copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I declare that the use of patient groups/records for this research maintains full patient confidentiality, and has undergone approval process by the committee and head of Australian Capital Territory Health Research and Ethics Committee, The Canberra Hospital and Medical Record Department, The Canberra Hospital. I also declare no conflicts of interest.

Dr Joseph Do Woong Choi

December 8th, 2017

Acknowledgements

- My family: Mother, father and sister, who continue to be my biggest supporters in life, as well as in completing this thesis;
- Professor Guan Chong: My mentor who has encouraged, motivated and inspired me to undergo surgical training and research, and helping me with study design, and thesis preparation;
- Dr Gavin Carney: My co-author of an international publication resulting from my thesis, who has provided guidance and expertise from a nephrology perspective;
- Dr Tack Tsiew Lee; My co-author of an international publication resulting from my thesis, who has provided guidance and expertise from Otolaryngology, Head and Neck Surgery;
- Dr Xiao Ming Liang: My associate supervisor, who has been a significant mentor both as a surgeon and as a researcher;
- Associate Professor Peter Hickman: For providing expert advice, feedback and information on biochemical assays from a chemical pathologist;
- Dr Michael Wilson: For providing expert advice and feedback from an anaesthetist;
- Associate Professor Girish Talaulikar: For providing expert advice, and feedback from nephrology;

- Ms Gloria Spiropoulos: For being an exceptional aide to data collection by promptly enabling access to medical records and for her support within the Canberra Hospital Medical Records Department.

Thesis Abstract

Background

Since the 1850s, parathyroid surgery continues to evolve through improved understanding of the pathophysiology. Dialysis dependant end stage renal failure (ESRF), the major cause of secondary hyperparathyroidism continues to rise in the western world. Other than renal transplantation, parathyroidectomy may provide a substantial cure for longstanding renal hyperparathyroidism in dialysis dependant patients. In 2004, cinacalcet was introduced as an alternative to the surgical management of renal hyperparathyroidism. However, cinacalcet was withdrawn from Australia's Pharmaceutical Benefits Scheme (PBS) in 2015, as the EVOLVE study failed to demonstrate a statistically significant reduction in the time to death, or non-fatal cardiovascular events in those treated with cinacalcet with renal hyperparathyroidism. This led to a re-emergence in parathyroidectomy. Additionally in our institution, patients who had been on cinacalcet, and subsequently underwent parathyroidectomy because of refractory disease or intolerance to cinacalcet, were noted to experience greater hyperkalaemia and hypocalcaemia in the intraoperative and immediate postoperative period.

Aims

- To review the engrossing history of the discovery and progression of parathyroid surgery since the 19th century;

- To correlate the embryology, anatomy, histology, physiology and pathophysiology of parathyroid glands in end stage renal failure;
- To provide up to date review in regards to investigation and the surgical management of renal hyperparathyroidism;
- Conduct a cohort study on the association of cinacalcet use with greater likelihood of intraoperative and immediate postoperative hyperkalaemia and hypocalcaemia following parathyroidectomy in renal hyperparathyroidism.

Methods

Literature reviews utilizing MEDLINE and Cochrane review databases, life science journals and textbooks were utilized. Hospital medical records from The Canberra Hospital were studied to collect data on the cohort case series. Analysis of data was performed using SPSS Statistics and Microsoft Excel.

Results

Sir Richard Owen is reputed to be the first person to discover the existence of parathyroid glands when examining a rhinoceros in 1852. In the spirit of *mortui vivos docent*, Captain Charles Martell in the 1930s had significantly increased our understanding of the existence of ectopic locations of parathyroid glands, as well as operative planning. The physiology and pathophysiology of parathyroid glands in chronic renal failure is

multifaceted, with a complex interplay between bone, kidneys, intestine, vitamin D, potassium, phosphate and magnesium. There are a range of investigative strategies for localizing parathyroid glands, often yielding greater sensitivity and specificity when utilizing a combination of imaging tools. The choice of operative strategy for parathyroidectomy is often influenced by surgeon's preference and the institution's resources, due to paucity of good randomized trials and meta-analysis. Finally, our cohort study has shown that prior cinacalcet use was linked closely with severe intraoperative and immediate postoperative hyperkalaemia, and greater hypocalcaemia compared to control patients who underwent parathyroidectomy for renal hyperparathyroidism.

Conclusions

The continued inquiry into the basic sciences around renal hyperparathyroidism ensures that we are able to question traditional protocols, and practice the best evidence based medicine. From this, cinacalcet emerged to change the medical and surgical landscape in the treatment of hyperparathyroidism. The results of the cohort study led to development of a protocol for the perioperative management of renal hyperparathyroidism in cinacalcet treated patients requiring parathyroidectomy.

Table of Contents

Publications and Presentations From Thesis

- Chong GC, **Choi JDW**, Lee TT. Carney G. Intraoperative and postoperative hyperkalaemia after total parathyroidectomy following exposure to cinacalcet in sixteen patients for renal hyperparathyroidism. *Clinical Otolaryngology 2017;* doi: 10.1111/coa.12880. [Epub ahead of print]
- **Choi JDW:** Cinacalcet associated acute potassium rise following parathyroidectomy for renal failure. *General Surgeons Australia Annual Scientific Meeting (GSA-ASM), Perth, Oral Presentation, 2014*
- **Choi, JDW:** Cinacalcet associated acute potassium rise following parathyroidectomy for renal failure. *RACS Annual Scientific Meeting, Canberra, ACT, Oral Presentation, 2014*
- **Choi, JDW:** Predictive markers for hungry bone syndrome following parathyroidectomy. *RACS Annual Scientific Meeting, Canberra, ACT, Oral Presentation, 2013.*

“Better is possible. It does not take genius. It takes diligence. It takes moral clarity. It takes ingenuity. And above all, it takes a willingness to try”

- Atul Gawande

PART II: LITERATURE REVEW OF PARATHYROID GLANDS AND HYPERPARATHYROIDISM IN END STAGE RENAL FAILURE

Chapter 1: History of the Parathyroid Glands and Parathyroidectomy

The discovery of parathyroid glands and the introduction of parathyroidectomy (PTX) has a rich historical background and involved medical doctors and scientists of renown. It also uniquely paralleled the history of surgery in general, initially influenced by observations of anatomists, pathologists and surgeons in Europe, and followed by contributions of several American teams. There is every reason to believe that diseases of the parathyroid glands affected occasional individuals in every period and culture in the history of mankind (Eknoyan, 1995). Although associated clinical features were described in early medical literature prior to the discovery of the parathyroid glands, their presence remained unnoticed until the mid-19th century. In fact, the parathyroid glands are credited to be the last mammalian organ discovered to be visible to the naked eye (Delbridge and Palazzo, 2007). The mechanisms involved in hyperparathyroidism were not fully appreciated until the early-20th century, and were a classic example of the slow progression of endocrinology by trial and error via laboratory investigation, and the examination of individual cases (Giddings et al, 2009). The first intentional PTX with curative intent was largely acclaimed to occur in 1925,

and from then on, the field of PTX started to be defined, and continually be refined till the present day.

In 1834, the Zoological Society of London purchased its first Great Indian Rhinoceros (*Rhinoceros unicornis*) for 1000 guineas. Following the animals' death in 1849, Sir Richard Owen **(Figure 1)**, Hunterian Professor and Conservator of the Royal College of Surgeons of England was given the uncommon opportunity to dissect the animal, which took place in the winter months of 1849 to 1850 at the Conservator's resident quarters (Modari et al, 2004).

Figure 1. Sir Richard Owen (1804-1892), British Comparative Anatomist (Roher and Schulte, 2007)

In his paper on the dissection in 1850 to the Zoological Society and later published in their *Transactions* in 1852, he described "a small, compact yellow glandular body that is attached to the thyroid where veins emerge", subsequently known as the Glands of Owen (Rogers-Stevane and Kauffman, 2008). The original preparation remains preserved in the Hunterian Museum at the College, consisting of part of the larynx, trachea and thyroid of the rhinoceros, with a parathyroid gland attached measuring 30×14×8cm to the upper extremity of the thyroid gland **(Figure 2)**. This probably was the first description of the parathyroid glands. Afterwards, there were unconfirmed

reports of ‘parathyroid glands’ in humans made by Robert Remark in 1855 and by Rudolf Virchow in 1863 (Rolleston, 1936).

Figure 2. Sir Richard Owen’s original Indian rhinoceros specimen demonstrating the parathyroid gland (in arrow). Courtesy of Royal College of Surgeons of England, London

Full credit was given to Swedish born Ivar Victor Sandstrőm **(Figure 3)**, a 25 year old medical student for identification, histologic description and naming of parathyroid glands, albeit claiming they were undeveloped embryologic portions of the thyroid gland. He dedicated three of his postgraduate years as a prosector in the Department of Anatomy in Upsala, Sweden to dissect parathyroid glands from 50 human cadavers and other mammals (cats, dogs, horses, rabbits and ox) (Eknoyal, 1995). He described the colour, protean shapes and positions of the gland, and performed microscopic studies on fresh autopsy specimens using several staining techniques. He named them *“glandulae parathyroidae”.*

Figure 3. Ivar Victor Sandstrőm (Roher and Schulte, 2007)

In the *Upsala Läkarefőrenings Főrhandlinger* 1880, he stated:

'I encountered on the thyroid of a dog a small hardly hemp-seed sized structure which was included in the same capsule as the thyroid, but distinguished itself from it by a brighter colour. A superficial examination revealed an organ of a structure entirely different from that of the thyroid and particularly amply vascularized, because of which I considered it probable that here a vascular gland had been encountered, analogous to the carotid glands' (Gley, 1891).

Sandstrőm was also aware of the variability of their location, and further observed:

"Although the glands were generally united with the thyroid by means of soft connective tissue, they were often movable against its capsule. Many of the glands are well defined fat lobules separated from the thyroid gland capsule. To each gland, there are one or more small arteriole branches from the inferior thyroid artery, and in the interstitial tissue there are often considerable fat cells and may be so numerous that the parenchyma of the gland appears only here and there in the spaces between the fat cells" (Sandstrőm, 1880).

The significance of Sandstrőm's discovery went relatively unnoticed internationally. His manuscript, with carefully detailed gross and microscopic drawings was rejected by famous German editors because of its

length. Later, his work was published in a local Swedish medical journal, *Upsala Läkaref̋renings F̋rhandlingar*. Sandstr̋m did not receive the acclaim he deserved during a short lifetime, later committing suicide (Dolev, 1987).

The contracture of the extremities, with or without peculiar seizures was detailed over 150 years ago when Corvisard introduced the term “tetany” for the first time in 1852 (Toneto et al, 2016). Trousseau, in 1862 and Chvostek, in 1876 defined specific clinical signs that characterize it, without defining its origin (Toneto et al, 2016). The combination of these tetanic symptoms was first described by Anton Wolfer in 1879, in a patient undergoing total thyroidectomy by Billroth (Toneto et al, 2016). In 1891, Eugene Gley, Professor of Physiology at the College de France in Paris, observed that when he injured animal parathyroids while leaving the thyroid intact, he observed tetany and reported a possible connection between PTX and tetany. His report was well received by European thyroid surgeons, and his results confirmed by Giulio Vassale and Francesco Generali in 1897, who found that extirpating both the thyroid and parathyroid glands in animals caused a milder clinical syndrome and less rapidly fatal outcome than was true for simple PTX (Dolev, 1987). Their conclusion was that after PTX alone, the animals were poisoned, believing the function of parathyroid glands was to detoxify toxins, however with thyroidectomy added to the surgery, the

metabolic rate decreased and this caused a decreased production of toxins (Idem, 1900). This was the basis for the detoxification theory which remained accepted until the mid-1920s (Vassale and Generali, 1896).

In 1891, Frederick von Recklinghausen reported a series of patients with bone disease demonstrating pathological fractures, brown tumours and skeletal cystic disease. This group of patients were subsequently termed 'osteitis fibrosa cystica', and although von Recklinghausen made an important clinical observation, he did not correlate this bone disease with hyperparathyroidism (Modarai et al, 2004).

The relationship between serum calcium, parathyroid glands and tetany was not fully understood until the early 1900s. William MacCallum is credited to be the first to convincingly demonstrate a link between hypocalcaemia and tetany in 1909 (Dolev, 1987). MacCallum and Carl Voegtlin demonstrated that post-parathyroidectomy tetany could be corrected by a parathyroid extract or by injection of calcium, but not potassium or sodium. They further demonstrated a decrease in tissue calcium in tetany, hyperexcretion of calcium in the urine or faeces, and increased urinary excretion of nitrogen (Dolev, 1987). In his classic experiment, MacCallum used dialysis to remove calcium from blood, injected it into tetanic animals, and observed that these animals remained tetanic when compared with animals given normal blood. He concluded that:

'The tetany of parathyroidectomy…is due to lack of calcium' (MacCallum et al, 1914)

Despite his authoritative statement, it was not universally accepted until much later in the 20th century, due to the popularity of the detoxification theory (Giddings et al, 2009). The first to suggest a connection between parathyroid gland disease, and diseases of the bone was from the work of Viennese pathologist Jacob Erdheim. He noted parathyroid hyperplasia in three patients with osteomalacia, and in a series of experiments in 1906, he selectively damaged rat parathyroid glands with cautery and observed tetany, as well as poor calcification of rat teeth (Eknoyan, 1995, Giddings et al, 2009, Rogers-Stevane and Kauffman, 2008). He went on to describe that this demineralisation of teeth could be reversed by allografting parathyroid tissue. Erdheim concluded the changes in the parathyroid glands were a compensatory response to the bone disease and thus beneficial (Eknoyan, 1995, Giddings et al, 2009). His work unfortunately led to great misinterpretation in the scientific community at the time, where the treatment of cystic bone lesions found on early radiographs was to supplement patients with parathyroid extract, or transplanting bovine parathyroid tissue to alleviate the "gap" between hormone production and demand (Rogers-Stevane and Kauffman, 2008). This misinterpretation was also enhanced by the widely accepted detoxification theory of the parathyroid glands at that

time. In 1925, James B Collip, a renowned Canadian biochemist successfully patented an extract of parathyroid hormone (PTH) from crushed ox parathyroid glands, and 'Collip's [parathyroid] extract' was mistakenly known to be the mainstay treatment for osteitis fibrosa cystica (Eknoyan, 1995, Thompson, 1990). Collip was also the first to induce experimental hypercalcaemia and to describe the effects of severe hypercalcaemia (Thompson, 1990).

Felix Mandl **(Figure 4)**, a Viennese surgeon and protégé of von Eiselberg, is acclaimed to be the first parathyroid surgeon. In 1925, he pioneered multiple surgeries seeking a parathyroid tumour, and excising it from the neck of a patient (Delbridge and Palazzo, 2007, Giddings et al, 2009, Rolleston, 1936). The patient's name was Mr Albert Gahne, who was a 34 year old tram car conductor whose symptoms dated back to 1921. He initially presented to von Eiselberg's clinic with hip and lower extremity pain. Mandl was involved in the patients' care and on x ray, diagnosed the patient as having osteitis fibrosa cystica. His blood and urinary calcium levels were found to be elevated, with an observed white urinary precipitate (Dolev, 1987). Initially, Mandl unsuccessfully treated Gahne's condition by administering thyroid extract, Collip's extract, and even attempted placing parathyroid graft from a donor victim of a road accident. Mandl then decided to explore the patient's neck and resected an enlarged parathyroid gland (25×15×12mm) with excellent bone recovery, normalisation of serum

calcium and decreased urinary calcium excretion (Rogers-Stevane and Kauffman, 2008). However, the patient developed recurrent disease and in 1933, Mandl re-operated on Mr Gahne's neck, and found two normal parathyroid glands, one of which was intra-thyroidal. A subtotal thyroidectomy was performed as he considered an intrathyroidal adenoma as a possibility; however the patient died three years later without postoperative tetany (Giddings et al, 2009). Given that the post-mortem did not identify any parathyroid tissue or tumour, it is suggested that the patient may have deceased from parathyroid carcinoma with occult metastasis (Carney, 1996).

Figure 4. Felix Mandl (1892-1957) was Professor of Surgery and Chairman of the Department of Surgery at Franz-Joseph-Spital, Vienna, Austria (Roher and Schulte, 2007)

However, Delbridge and Palazzo (2007) considered that Sir John Bland-Sutton, President of the Royal College of Surgeons of England, UK 1923 had performed the first intentional removal of a parathyroid tumour before 1917, almost a decade before Mandl. Bland-Sutton was a pre-eminent surgical pathologist of his time. He had extensive experience of parathyroids where he described a post-mortem specimen of a parathyroid tumour in 1886 and surgically removed a parathyroid cyst in 1909 (Delbridge and Palazzo,

2007). In Bland-Sutton's sixth edition (1917) of the textbook *Tumours: Innocent and Malignant*, he recounts one of his patients:

'…a young married woman…was on a ship in the Red Sea and had great difficulty in breathing; a small rounded lump was detected in her neck below the thyroid gland. It increased in size and the dyspnoea became so urgent that one night the patient was prepared for tracheostomy and the ship's surgeon remained by the bedside with instruments. Fortunately, the swelling subsided. On her return to England, I removed the rounded body, as big as a cherry, situated below the lower angle of the thyroid gland on the left side of the trachea. It had the microscopic features of a parathyroid' (Bland-Sutton, 1917).

The report could be consistent with a haemorrhage into a parathyroid adenoma. It is unclear whether the presentation in this case was of mechanical effects of the parathyroid adenoma with local pressure, or whether the patient suffered symptoms associated with a hypercalcaemic crisis given that the removed tumour was only the size of a cherry (Delbridge and Palazzo, 2007). However, given the paucity of knowledge regarding associations of calcium, bone disease and parathyroid gland during this time, Mandl must still be credited for carrying out his operation with a curative intent on removing an abnormal parathyroid gland (Delbridge and Palazzo, 2007).

Cervical explorations for pathological parathyroid glands became increasingly popular and refined after Mandl, however surgeons were grappling with the possibility and difficulty of ectopic parathyroid glands. Captain Charles Martell **(Figure 5a)**, a 30 year old male of New York City was a healthy marine sea captain until three years before admission, when generalized skeletal decalcification set in (Dolev, 1987). He was investigated at the Massachusetts General Hospital (MGH) to have serum calcium and phosphorus levels of 14.8mg/dL and 3.3mg/dL respectively. A diagnosis of primary hyperparathyroidism was confirmed and in May 1927, Captain Martell underwent the first two operations by EP Richardson (Chief of Surgery, MGH) through a collar incision; the right neck was explored without finding an adenoma. A single normal parathyroid gland was removed. The second operation was limited to the left side, only one normal parathyroid gland was found (Dolev, 1987). In 1929, Captain Martell was re-hospitalized where he underwent a third failed neck exploration. Unfortunately, his symptomatology was increasing, and renal function was deteriorating. He was readmitted to MGH in 1932, where he was studied extensively for 18 months in two metabolic wards. During this period, Oliver Cope **(Figure 5b)**, under the direction of Edward Churchill **(Figure 5c)** (new Chief of Surgery, MGH) carried out a series of parathyroid gland dissections in cadavers in preparation for Martell's re-operation. Cope had a reputation of performing several successful parathyroidectomies in early 1932 (Dolev,

1987). During the latter half of 1932, Cope (who was assisted by Churchill) performed three additional negative cervical explorations. Meanwhile, Captain Martell read extensively in the Harvard Medical Library about the various locations of parathyroid glands. He found in the December 1931 issue of *Acta Medica Scandinavica* that described a mediastinal parathyroid adenoma, and managed to convince Cope and Churchill to explore his mediastinum. In Martell's seventh operation, a 3.0 cm mediastinal adenoma was found (Rogers-Stevane and Kauffman, 2008, Ellis, 1983). Churchill and Cope excised only 90% of the adenoma, attaching the remnant with its vascular pedicle to tissue in the region of the external notch. Despite these precautions, tetany developed on the third postoperative day (Dolev, 1987). Six weeks after the operation, a ureteric calculus became impacted. Captain Martell died from laryngospasm shortly after surgery to relieve obstruction from his stone. Few patients in the annals of history have been studied as extensively as Captain Martell (Dolev, 1987).

Figure 5. Top left and right: Captain Charles Martell; Bottom left: Oliver Cope; Bottom right: Edward Churchill (Roher and Schulte, 2007)

Benjamin Castleman (MGH) and others began to document gradually larger series of PTX in the 1930s, and the realization that the pathology of primary hyperparathyroidism was associated with not only solitary adenoma, but also

double adenomas and multi-glandular hyperplasia was soon recognized (DuBose and Morvant, 2005). Thus, bilateral neck explorations proved to be a commonly used procedure until more minimally invasive procedures began to gain acceptance in the 1980s.

Pappenheimer and Wilens in 1935 were one of first authors to report that parathyroid glands from patients with chronic nephritis were significantly larger than glands from normal individuals, due to 'some common chemical factor that stimulates the parathyroids to increased activity and growth' (Pappenheimer and Wilens, 1935). They concluded that the cases involving severe nephritis were characterized by phosphate retention, which would result in a reduction in ionized calcium (as determined by Collip). This may incite the parathyroid glands to secrete more parathyroid hormone and grow in size (Pappenheimer and Wilens, 1935). At about this time, William Kloff, a Dutch physician, considered the Father of Dialysis, construct the first renal dialyzer after helplessly watching a young man die slowly of renal failure at the University of Groningen Hospital, Netherlands in the late 1930s (Davita, 2017). He was inspired by a report from a pharmacologist, John Abel in 1913, from the Johns Hopkins University, who reported on haemodialysis in animals (Davita, 2017). In 1943, Kloff constructed his first haemodialysis machine with sausage skins, orange juice cans, and used auto parts, and treated 16 patients with acute renal failure, initially with little success

(Davita, 2017). After continued improvisation, he was able to regain consciousness of a 67 year old woman in uraemic coma using Kloff's dializer (Davita, 2017). Her first words were reportedly "I'm going to divorce my husband!" (Davita, 2017). This paved way for new understanding of renal hyperparathyroidism, as patients with end stage renal failure survived longer with dialysis, and developed more florid secondary hyperparathyroidism.

After the initial findings of Collip's extract, laboratory measurements for PTH were extremely unreliable, hampering its applicability. It took another 40 years before the discovery of a more effective method for the measurement of PTH and other peptides by Berson and Yalow, giving rise to more precise evaluation of such patients (Toneto et al, 2016). They developed a radio-immunoassay analytical test, which won them a Nobel Prize in 1977 (Nobelprize.org, 1977). The improvement in the determination of serum calcium and PTH yielded an improvement in the understanding of metabolic bone and calcium related disorders. The number of patients diagnosed with hyperparathyroidism, even asymptomatic, increased considerably, making it possible to uncover the various clinical and metabolic aspects related to diseases of the parathyroid glands (Toneto et al, 2016). Berson and Yarlow's discovery facilitated a significant increase in parathyroid operations during the 1980s.

In 1982, Tibblin et al proposed unilateral neck exploration and parathyroidectomy for primary hyperparathyroidism as an acceptable alternative, provided that the normal ipsilateral gland could be identified (DuBose and Morvant, 2005, Tibblin et al, 1982). Better preoperative and intraoperative localization studies supported the use of this surgical option and even less invasive procedures in some situations (DuBose and Morvant, 2005). In 1989, AJ Coakely noticed that technetium sestamibi was rapidly taken up by the parathyroid glands, thus provided surgery with a potent tool for preoperative imaging, useful in both initial and redo surgeries (Coakley et al, 1989). In 1988, Nussbaum provided evidence that PTH could be measured rapidly during the operation and thereby confirming success, hence rendering intraoperative frozen section much less important (Nussbaum et al, 1998). Different combinations of imaging techniques and intraoperative hormone assessments are still under consideration with regard to successfully localizing glands, and increasing cost efficiency. The use of endoscopic parathyroidectomy for parathyroid disease of the mediastinum by Prinz et al at Rush University Medical Centre, and in the neck by Gagner at Columbia in the mid-1990s has introduced exciting additional surgical options for consideration (Prinz et al, 1994, Gagner, 1996).

In summary, the history of parathyroid glands and parathyroidectomy is one that has initially confused and misled clinicians and patients over the last 180 years. Its understanding of diseases has developed through chance, trial and error, case reports and laboratory research. Contributors have come from various fields of science, and have made important contributions at various stages of their training. There is great debt to the early sufferers of parathyroid diseases for their help in advancing our understanding. It is prudent to remember the evolution that has already occurred, even as medicine continues to write new chapters in the history of parathyroid surgery.

Chapter 2: Embryology, Anatomy and Histology of Parathyroid Glands

2.1 Embryology

The parathyroid glands develop from the pharyngeal pouches, which are temporary endodermal out-pockets that also form the thymus and ultimo-branchial bodies in vertebrates. The pharyngeal apparatus consists of the pharyngeal arches, pouches, grooves and the membranes. These early embryonic structures contribute to organogenesis of the head and neck structures.

The pharyngeal arches begin to develop early in the fourth week of gestation, as neural crest cells migrate into the future head and neck regions. Each pharyngeal arch consists of a core of mesenchyme and is covered externally by ectoderm, and internally by endoderm (Moore et al, 2013). There are five pharyngeal arches (of which the fifth pharyngeal arch is rudimentary), each separated by a pharyngeal groove and they contribute extensively to the development of the face, nasal cavities, mouth, larynx, pharynx and neck (Moore et al, 2013).

The endoderm of the pharynx (that lines the internal aspects of the pharyngeal arches) passes into pharyngeal pouches, which develop in a cranio-caudal sequence between the arches (Moore et al, 2013). The

pharyngeal membranes are double layered, and derived from the endoderm of pouches which contacts the ectoderm of the pharyngeal grooves. There are four defined pairs of pharyngeal pouches in humans (fifth pair is rudimentary), each which gives rise to important organs of the head and neck.

The development of the parathyroid glands can be thought of as being intimately connected with that of the thymus (particularly the inferior parathyroid glands), as it is often found by the occurrence of the parathyroid gland(s) embedded on the surface of the thymus, or buried within its substance (Gilmour and Grocers, 1937). They originate from the third (inferior parathyroid glands) and fourth (superior parathyroid glands) pharyngeal pouches that also form the thymus and ultimo-branchial bodies in vertebrates **(Figure 6)**.

Figure 6. Schematic of the branchial pouch and arches. The superior parathyroid glands originate from the fourth branchial pouch along with the ultimobranchial body that will contribute to the development of the thyroid gland. The inferior parathyroid glands originate from the third branchial pouch along with the thymus (Policeni et al, 2012).

Nearly all of the information regarding parathyroid organogenesis has come from studies in mice, facilitated by the identification of the early regulation

of parathyroid differentiation genes, *glial cells missing 2,* or *Gcm2* (Manley, 2015, Kim et al, 1998). The expression of *Gcm2* throughout parathyroid organogenesis allowed the tracking of parathyroid fated cells during embryonic development and has been a key to the recent developments in understanding parathyroid organogenesis (Manley, 2015, Kim et al, 1998). As development proceeds, genes involved in calcium homeostasis, such as parathyroid hormone (PTH), and calcium sensing receptor (CasR) are upregulated in the developing parathyroid, and with *Gcm2,* remain transcribed in the parathyroid rudiments as they migrate towards the midline and into adulthood (Liu and Manley, 2007).

The third pharyngeal pouch expands and develops a solid, dorsal bulbar part and a hollow, elongate ventral part. By the sixth week of gestation, the epithelium of each dorsal bulbar part of the pouch begins to differentiate into an inferior parathyroid gland, whilst the epithelium of the elongate ventral parts of the pouch proliferates, to form the thymus (Moore et al, 2013). The thymus and parathyroid glands both usually lose their connections to the pharynx at seven weeks gestation (Zapanta and Meyers, 2016). The thymus migrates caudally and medially, pulling the inferior parathyroid glands with it, therefore they are positioned in a more inferior position than are parathyroid glands derived from the fourth pharyngeal pouch (Zapanta and Meyers, 2016). Later, the parathyroid glands separate from the thymus to lie on the dorsal surface of the thyroid gland, usually outside of the fibrous

capsule of the gland itself. This close relationship between the inferior parathyroid glands and the thymus explains the variable position of the inferior parathyroid glands, as the thymus has a long course of descent into the superior mediastinum, and frequently presents in ectopic locations (Policeni et al, 2012) (see section on anatomy of parathyroid glands). The fibroblast growth factor signalling pathways, acting through FRS2-α are thought to be implicated in the development of the thymus and parathyroid glands (Moore, 2013, Manley, 2015, Grevellec and Tucker, 2010).

The superior parathyroid glands originate from the fourth pharyngeal pouch. The fourth pharyngeal pouch expands into dorsal bulbar and elongate ventral parts, and its connection with the pharynx is reduced to a narrow duct that soon degenerates (Moore et al, 2013). The superior parathyroids start developing from the dorsal part and migrate caudally during the fifth and sixth weeks of gestation (Policeni et al, 2012, Moore and Persaud, 1998, Safford and Skinner, 2006). At gestational week seven, the glands lose connections with the pharynx and attach themselves to the thyroid gland, which is also migrating caudally. The superior parathyroids are generally located more posterior and medial than the inferior parathyroid glands, and their final resting point is usually on the dorsal surface of the thyroid gland, outside the fibrous capsule of the thyroid gland (Zapanta and Meyers, 2016).

2.2 Anatomy

The parathyroid glands are small flattened, oval/kidney bean shaped bodies which usually lie on the medial half of the posterior surface of each lobe of the thyroid gland, where they may be either within, or outside the thyroid capsule of pre-tracheal fascia (Moore et al, 2010, McMinn, 2011). Most humans have four parathyroid glands (two superior, two inferior), although this can range from two to six glands (supernumerary), with some authors reporting up to twelve glands (Som and Curtin, 2003, O'Rahilly and Muller ,1996) **(Figure 7)**. In one series, more than four glands were found in 13% of the cases, four glands in 84% and three glands in 3% of the cases (Akerstrom et al, 1984). The percentage of individuals with less or more than four glands may be difficult to report as the researcher may have been unsuccessful in finding one or more glands, and a missed gland could represent an unobserved rather than an absent gland (Herrera and Gamboa-Dominguez, 2005).

Figure 7. Posterior view of the pharynx, oesophagus and thyroid glands to visualize superior and inferior parathyroid glands (Mohebati and Saha, 2012)

The colour of normal parathyroid glands varies depending on the age of the patient, extracellular fat content, the degree of vascular congestion, and the number of oxyphil cells present. In the newborn, they are grey and semitransparent. They are light pink in children, turning yellow in adults as their fat content increases. In older adults, they become darker (Herrera and Gamboa-Dominguez, 2005). The parathyroid glands may conceivably be confused with small lobules of adipose tissue, with accessory nodules of the thyroid gland, or even with lymph nodes (Greenson et al, 2015). Normal glands are usually the size of a grain of rice or lentil, between 2 and 7mm in length, 2 and 4mm in width, and 0.5 to 2mm in thickness. The combined weights of all parathyroid glands in a healthy male averages about 120mg, and in an adult female around 145mg. Weights of individual glands range from 30 to 75mg, with reported averages of 35 to 55mg (Greenson et al, 2015).

The superior gland originates from the fourth branchial pouch, and as parathyroid glands lose their attachment with the pharyngeal wall, they attach to the posterior mid surface of the caudally migrating thyroid, near the tracheal-oesophageal groove (Mohebati and Saha, 2012). It has a shorter embryologic descent than their inferior counterparts, which leads to a smaller variability in location **(Figure 8)**. In 85% of cases, the superior parathyroid can be found at the posterior aspect of the thyroid lobe in a two centimetre area centred one to two centimetres cranial to the crossing of the inferior

thyroid artery and the recurrent laryngeal nerve (Fancy et al, 2010, Randolph, 2003). Where the recurrent laryngeal nerve enters the point of Ligament of Berry and behind the cricoid cartilage, the superior glands may be found within 1cm. They are usually at the level with the first tracheal ring, and lie under the thyroid superficial fascia, posterior to the recurrent laryngeal nerve. They may also reside inside the thyroid capsule, just superior and medial to the posterior tubercle of Zuckerkandl of the thyroid (Richards et al, 2015). In an autopsy series of 503 cases, 2% of ectopic superior parathyroid glands were found at the level of superior pole of the thyroid, and less than 1% were superior to it (Akerstrom et al, 1984, Fancy et al, 2010). They may also be placed further down, sometimes obscured by the inferior thyroid artery and the recurrent laryngeal nerve. Rarely, they are found in the retro-pharyngeal space, retro-oesophageal space and intra-thyroidal (Herrera and Gamboa-Dominguez, 2005).

Figure 8. Variations in the position of the superior and inferior parathyroid glands (Herrera and Gamboa-Dominguez, 2005)

The inferior parathyroid glands have a more variable location due to their embryologic relationship to the thymus, which are both derived from the third branchial pouch (Policeni et al, 2012) **(Figure 8)**. In 50% of cases, they are found within one centimetre inferior, lateral or posterior to the lower pole

of the thyroid, and are typically anterior to a plane drawn along the course of the recurrent laryngeal nerve and the superior parathyroid glands (Fancy et al, 2010, Randolph, 2003). However, ectopic inferior glands can be found anywhere along a large area of descent from the angle of the mandible to the superior border of the pericardium and the aorto-pulmonary window (Gray et al, 1976) **(Figure 9).** In a study of 645 parathyroid glands from 160 cases, 42% of the inferior parathyroid glands were found on the inferior or the postero-lateral surface of the lower pole of the thyroid, 39% were located in the lower neck in proximity to the thymus and thyro-thymic ligament, 15% lateral to the thyroid, and only 2% within the mediastinal thymic tissue (Mohebati and Shaha, 2012, Wang, 1976). In rare cases (2.8%), the inferior gland can be found superior to the intersection of the recurrent laryngeal nerve and the inferior thyroid artery, intra-thyroidal (3%) and within the carotid sheath (2%) (Herrera and Gamboa-Dominguez, 2005, Fancy et al, 2010). If there has been a failure of inferior gland descent during its embryological development, they may be found high in the neck and even above the upper pole of the thyroid; however these glands are usually surrounded by a remnant of thymic tissue (Herrera and Gamboa-Dominguez, 2005).

Figure 9. A-C: Coronal, sagittal and transverse Computed Tomography (CT) scan of head and neck and superior mediastinum, demonstrating enhancing

mass in superior mediastinum, just anterior to the aorta and posterior to the sternum. D: Tc-99m sestamibi scan demonstrating persistent uptake of superior mediastinum mass at 15(left) and 90 minutes (right) delay, consistent with a parathyroid adenoma (Policeni et al, 2012).

In the majority of cases, parathyroid glands are located in symmetrical position in the neck. There have been reports of symmetry at 80% for the superior and 70% for the inferior glands, with a relative symmetry of 60% for all four glands (Akerstron et al, 1984, Mohebati and Shaha, 2012, Fancy et al, 2010). Thus, when unable to locate a missing parathyroid, contralateral neck exploration for comparison may be useful.

Supernumerary parathyroid glands are known to occur in 2.5-15% of cases of which the majority are small, rudimentary or divided (Akerstrom et al, 1984, Carter et al, 1993). They may be found anywhere along the line of thymic tissue descent during embryological development, however in two thirds of cases, they are found within the thymus or in relation to thyro-thymic ligament. These glands may be responsible for persistent hyperparathyroidism after parathyroid surgery, especially in secondary and tertiary hyperparathyroidism, and in familial syndromes (Richards et al, 2015).

The blood supply of both upper and lower parathyroid glands is usually supplied by the inferior thyroid artery, with minute veins joining thyroid

vessels (McMinn, 2011). Each gland usually has its own end-artery. In particular, the superior parathyroid glands receive 80% of their arterial blood supply from this artery, 15% by the superior thyroid artery (most commonly via the posterior branch) and 5% via anastomoses running between the two systems (Herrera and Gamboa-Dominguez, 2005). On the other hand, 10% of the inferior parathyroid glands are vascularized by the superior thyroid artery, via anastomoses of both systems, or Neubauer's artery (Herrera and Gamboa-Dominguez, 2005, Flament et al, 1982). Ligation of the branches of the inferior thyroid artery close to the thyroid parenchyma and medial to the recurrent laryngeal nerve may help preserve intact parathyroid vascularity during thyroid surgery (Richards et al, 2015). The venous drainage of the parathyroid glands are tributaries of the superior, middle and inferior thyroid veins that drain into the internal jugular vein or the innominate vein.

The lymphatic drainage of the glands are as for the thyroid gland and the thymus, which parallels the venous drainage. Lymph that follow superior and middle thyroid veins drain into the upper deep nodes of the cervical chain, and those accompanying the inferior vessels drain to the lower nodes of the cervical plexus, supraclavicular, paratracheal and parapharyngeal nodes (Mohebati and Shaha, 2012, Hoyes and Kershaw, 1985).

The nerve supply of the parathyroid glands is abundant; it is derived from thyroid branches of the cervical sympathetic ganglia, which enter the

parathyroid parenchyma with the arteries. Like the nerves to the thyroid, they are vasomotor rather than secreto-motor because the glands are hormonally regulated (Moore et al, 2010).

Ultimately, the surgical implication is that appreciation and sound understanding of the embryology and anatomy of the parathyroid glands assist surgeons to better locate and visualize the glands, decreasing the incidence of missing an abnormal/ectopically located/supernumerary gland(s), conditions leading to persistence of disease.

2.3 Histology

2.3.1. Normal parathyroid gland

The parathyroid gland is enclosed within a thin connective tissue capsule **(Figure 10)**. Thin septa of loose connective tissue extend inwards and merge with the delicate framework of reticular fibres, carrying the blood vessels, vasomotor nerves and lymphatics (Junquiera et al, 1998). However, the septa do not divide the gland into distinct lobules (Ham and Cormack, 1989). From these septa, epithelial cells may rarely form isolated small follicles resembling the thyroid gland, containing colloid material bearing no functional relationship to the colloid of the thyroid gland. The connective tissue stroma of the gland, in normal adults, contains a variable number of adipose cells. Historically, the cell to fat ratio of 50:50 has been accepted as normal for adults, although in the elderly, the fat may

occupy as much as 60% of the gland (Greenson et al, 2015, Fawchett and Jensh, 2002). However, the overall weight of the parathyroid glands normally remains constant during adult life, thus there is an age-related decrease in the mass of chief cells (Kacsoh, 2000).

The parenchyma of the human parathyroid gland consists of three cell types: the chief (or principal) cells, the oxyphil cells and transitional cells. The latter cell's function however, is still yet to be elucidated (Junquiera et al, 1998) **(Figure 10)**.

Figure 10. Haematoxylin and eosin stain photomicrograph of human parathyroid gland. The connective tissue capsule (Cap) surrounds the gland with blood vessels (BV) located within the septum separating the lobes of the parathyroid gland. The principal cells are separated by oxyphil cells. Adipose tissue (AC) become more abundant with age (Ross and Pawlina, 2006)

Chief cells are small, polygonal cells with a diameter of 4 to 8 micrometres and a centrally located nucleus. They are the more numerous of the cell type, responsible for the secretion of PTH. Sometimes, they are divided into clear and dark forms, depending on the amount of granules in its cytoplasm. Its' cytoplasm is lightly eosinophilc, containing lipofuscin-containing vesicles, large accumulations of glycogen and lipid droplets (Ross and Pawlina, 2006). On electron microscope, they are found to be joined by occasional desmosomes (Fawchett and Jensh, 2002). In contrast to other glands, in

which all cells normally exhibit the same degree of activity, parathyroid chief cells appear to go through their secretory cycle independently. Amongst the active cells, they are seen to contain long mitochondria, parallel cisternae or rough endoplasmic reticulum, a prominent Golgi complex and small deposits of glycogen. Within the cytoplasm, small, dense, membrane-limited vesicles containing irregularly shaped granules 200-400 nanometre in diameter are thought to be the storage form of PTH (Junquiera et al, 1998, Ross and Pawlina, 2006). These granules are distributed throughout the cytoplasm; sometimes they are more numerous at the vascular pole of the cell (Junquiera et al, 1998).

On the other hand, oxyphil cells constitute a minor portion of the parenchymal cells, and are not known to have a secretory role. The precise functions of these cells are still uncertain. They begin to appear from about age 5-7, and increase in number with age, with some suggestion that they probably arise from chief cells previously present (Junquiera et al, 1998, Ham and Cormack, 1989). They are found singly or in clusters; the cells are more rounded, considerably larger (6-10micrometer in diameter) than chief cells, and have a distinctly acidophilic cytoplasm, which is granular (Ross and Pawlina, 2006, Leeson et al, 1985). They have small, darkly staining nuclei (Leeson et al, 1985). Their Golgi complex is small, the cytoplasmic reticulum is sparse, and there are no secretory granules. They have an unusually large number of mitochondria and have closely spaced cristae,

suggesting a high degree of metabolic activity (Fawchett and Jensh, 2002). Cytoplasmic inclusion bodies consist of occasional lysosomes, lipid droplets and glycogen distributed among the mitochondria (Ross and Pawlina, 2006).

Finally, the transitional cell is thought to have cytological characteristics intermediate between those of the chief cell and the oxyphil cell (Bloom and Fawcett, 1997). It stains with acid dyes and has a nucleus that is somewhat similar and more deeply staining than that of the other cell types. Because only one hormone is produced by the parathyroid glands, the above three cell forms are widely thought to be different phases in the life cycle of a single cell type, with the chief cell being its physiologically active stage (Bloom and Fawcett, 1997). This interpretation is supported by immunological studies localizing radioactive precursors of the hormone mainly over the secretory granules of the chief cells (Bloom and Fawcett, 1997).

2.3.2: Parathyroid glands in end stage renal failure

The vast majority of parathyroid gland abnormality in end stage renal failure is adaptive parathyroid hyperplasia, defined as an absolute increase in the mass of the parenchymal cells of the parathyroid gland. Most cases are chief cell hyperplasia. Usually, all four glands are involved, weighing on average, a total of 1-3 grams (Pathology Outlines, 2017). In advanced hyperplasia,

variations in gland size may be considerable, and the glands may exhibit considerable fibrosis, cyst formation and calcification (Fawchett and Jensh, 2002) **(Figure 11)**.

Figure 11. Cross sections of parathyroid glands demonstrating variations in sizes, colour and nodularity. R: Right parathyroid glands; L: Left parathyroid glands (Fawchett and Jensh, 2002, Roth, 1997).

The earliest change in the parathyroid glands is a reduction in the quantity of fat cells in the intervening stroma, and their partial replacement by vacuolated chief cells, arranged in either diffuse sheets, cord-like, acinai or trabecular growth patterns (Fawchett and Jensh, 2002, Greenson et al, 2015). Their nucleus is small and dense, and occupies an eccentric position within the cytoplasm (Fawchett and Jensh, 2002). Vacuolated chief cells are typically rich in glycogen, with little neutral lipid. Ultra structurally, they have fairly straight plasma membranes with few desmosomal attachment sites (Fawchett and Jensh, 2002). Their cytoplasm contain numerous secretory granules, adjacent to Golgi regions or plasma membranes (Fawchett and Jensh, 2002). Oxyphil cells are usually fewer in number (Greenson et al, 2015). Nodule formation is common in advanced renal hyperparathyroidism composed mainly of chief cells, which may be surrounded by fibrosis and for some, entirely encapsulated (Greenson et al,

2015, Lawrence, 1978) **(Figure 12)**. A rim of normal parathyroid tissue is not typically present, as is used as a criterion for the diagnosis of adenoma (Lawrence, 1978).

Figure 12. Multiple nodules can be seen surrounded by fibrosis in secondary hyperparathyroidism, with adjacent parathyroid tissue diffusely hyperplastic (Greenson et al, 2015)

Chapter 3: Parathyroid Gland Physiology and Calcium Homeostasis

The hormones involved with calcium homeostasis include (1) parathyroid hormone (PTH), a protein secreted by the parathyroid glands (2) Vitamin D-1, 25 dihydroxycholecalciferol (1, 25 DOHCC), a steroid; (3) calcitonin, a protein produced and released by the para-follicular cells of the thyroid gland; (4) parathyroid hormone related protein (PTHrP), particularly in paraneoplastic syndromes and (5) gonadal steroids (estrogens and/or testosterone) (Moffett et al, 1993).

3.1 Parathyroid Hormone

The PTH gene possesses upstream regulatory elements in the 5' region, including both vitamin-D and vitamin-A response elements. The vitamin-D response elements bind a vitamin D receptor (VDR), when the receptor is occupied by a vitamin D metabolite, usually 1, 25 DOHCC (Boron and Boulpael, 2012). The receptor has a very high affinity for 1, 25 DOHCC, less affinity for the 25-hydroxy form, and little affinity for the parent vitamin (either vitamin D2 or D3). Binding of the vitamin D-VDR complex to the VDR response element reduces the rate of PTH transcription (Boron and Boulpael, 2012). PTH is initially synthesized in the chief cells as a larger molecular weight precursor called pre-pro-parathyroid hormone (115 amino

acids). This then enters the endoplasmic reticulum, whereby a leader sequence is removed from the amino terminal to form the 90-amino acid polypeptide pro-PTH (Glasby and Huang, 1995). Six further amino acids are then cleaved before secretion from the secretory granules, such that the parathyroid hormone itself contains only 84 amino acids (Glasby and Huang, 1995) **(Figure 13).** Once secreted, PTH circulates free in blood plasma, and is rapidly metabolized; the half-life being approximately 4 minutes. Next, the circulating PTH is then cleaved into two principal fragments in the liver- a 33- or 36-amino acid N terminal peptide, and a larger C terminal peptide, with virtually all of the known biologic activity of PTH residing in the N-terminal fragment, which is rapidly hydrolysed, especially in the kidney (Boron and Boulpael, 2012). In the past, this cleavage made the interpretation of results of PTH assays confusing, since the N- terminal and C-terminal fragments cross-reacted in the PTH immunoassay. Newer assays now measures only the intact PTH hormone (i.e. 84 amino acids), and have allowed scientists to expand their understanding of PTH physiology (Glasby and Huang, 1995).

Figure 13. Molecular pathway of PTH synthesis. The production of PTH initially begins with pre-pro-PTH (115 amino residues) in the rough endoplasmic reticulum, which is then cleaved to form a pro-PTH (90 residues). In the Golgi apparatus, the pro sequence is cleaved by its enzymes to form PTH hormone stored in secretory granules (84 residues). This is then

further cleaved into two fragments, with the N terminal fragment (33-36 amino residue) containing all of the biologic activity (Boron and Boulpael, 2012)

PTH release is controlled in a tight feedback system by small changes in plasma calcium levels detected by the parathyroid calcium-sensing receptor (CaRs), a G protein-coupled receptor located on the plasma membrane of the parathyroid chief cells; it is also found in kidney tubule cells and thyroid C cells (Raff and Levitzky, 2011). This receptor binds calcium in a saturable manner, with an affinity profile that is similar to the concentration dependence for PTH secretion (Boron and Boulpael, 2012). Coupling of CaRs to Gαq activates phospholipase C, thus generating inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) and resulting in the release of calcium from internal stores and the activation of protein kinase C (Boron and Boulpel, 2012). However, unlike most G protein coupled receptors in which activation of these signalling systems promotes a secretory response, an inverse relationship between the concentration of ionized calcium in the extracellular fluid and PTH secretion by the chief cells exists, which is steeply sigmoidal. This means that a minor decrease in extracellular calcium leads to a marked increase in PTH secretion within seconds (Raff and Levitzky, 2011, Kacsoh, 2000). The inflection point of the negative sigmoid curve is found at an extracellular calcium slightly lower than the normal plasma value. Thus, under resting conditions, PTH is secreted at rates of only

2-25% of the maximal output (Kacsoh, 2000). In the chief cell, stimulus secretion coupling is performed by intracellular magnesium, rather than by calcium. Thus, depletion of magnesium stores can lead to hypoparathyroidism, as well as hyper-magnesemia can also inhibit PTH secretion due to its binding with the CaRs (Kacsoh, 2000).

PTH increases reabsorption of calcium in the distal tubules, although calcium excretion in the urine is often increased in hyperparathyroidism because the increase in the load of filtered calcium overwhelms the effect on reabsorption (Barrett et al, 2012). PTH increases the phosphate excretion in the urine and thereby, depresses plasma phosphate levels (Barrett et al, 2012). PTH also increases the formation of 1, 25 DOHCC by stimulating 1α hydroxylase activity in the proximal tubular cells of the kidney, and this further increases the calcium absorption from the intestine (Michael and Sircar, 2010). Finally, PTH acts directly on bone to increase bone resorption and mobilize calcium **(Figure 14)**.

Figure 14. The systemic effects of increased PTH from reduced plasma calcium concentration. PTH (along with synergistic effects with vitamin D) has effects on end organs (kidneys, bone, intestine) to counteract relative hypocalcaemia (Moffett et al, 1993).

A key action of PTH is to promote the reabsorption of calcium in the thick ascending limb (TAL) and distal convoluted tubule (DCT) of the kidney.

Most of the approximately 250 milli-moles of calcium filtered each day is reabsorbed in the proximal tubule (~65%) and TAL (~25%), with the distal nephron reabsorbing additional 5-10% of the filtered load of calcium (Boron and Boulpael, 2012). Thus, when PTH stimulates distal calcium reabsorption, it will greatly decrease the amount of calcium excreted in the urine. Vitamin D has a synergistic action of promoting calcium reabsorption in the DCT (Boron and Boulpael, 2012). Concurrently, PTH promotes phosphate excretion into the urine by inhibiting the reabsorption of phosphate in the proximal and distal tubule, resulting in phosphaturia and hypophosphatemia (Barrett et al, 2012, Boron and Boulpel, 2012). This phosphaturia results from a PTH-induced redistribution of the Na/phosphate co-transporter (NaPi) away from the apical membrane of the renal proximal tubule, and into a pool of subapical vesicles for subsequent lysosomal degradation (Raff and Levitzky, 2011, Boron and Boulpael, 2012). The elimination of phosphate is physiologically important, as PTH induced increase in plasma calcium, if accompanied by a rise in phosphate levels from degradation of bone, would cause phosphate salts to precipitate out of the soluble phase, at least partly negating the action of PTH to raise plasma calcium levels (Boron and Boulpael, 2012). Thus, the two functions of calcium retention and phosphate excretion tend to leave plasma $[Ca^{2+}]$▪[Pi] unaltered (Kacsoh, 2000).

PTH has multiple actions on bone, some direct and others indirect, of which the changes mediated by PTH are observed within minutes. The chronic effects of PTH are to increase the number of bone cells, both osteoblasts and osteoclasts, and to increase the remodelling of bone- these effects are apparent within hours after the hormone is given and persist for hours after PTH is withdrawn (Kasper et al, 2005). Continuous exposure to elevated PTH (such as in renal hyperparathyroidism) leads to increased osteoclast-mediated bone resorption. These processes are due to PTH binding onto receptors found in osteoblasts stimulating the activity of several proteins, including osteoclast-differentiating factor (ODF), also known as receptor activator of nuclear factor-κβ ligand (RANKL), or osteoprotegerin ligand (Raff and Levitzky, 2011). In addition, PTH stimulates osteoblast expression of genes involved in degradation of the extracellular matrix and bone remodelling (collagenase-3), production of growth factors (insulin like growth factor 1), and stimulation and recruitment of osteoclasts (RANKL and interleukin 6) (Raff and Levitzky, 2011).

Interestingly to a much lesser extent, *intermittent* administration of PTH, elevating hormone levels for 1-2 hours each day, leads to a net stimulation of bone formation, rather than breakdown (Kasper et al, 2005). In this pathway, PTH promotes bone synthesis directly by activating calcium channels from bone fluid to the osteocytes. The osteocytes then transfers the calcium via

gap junctions to the osteoblasts at the bone surface, a process called osteocytic osteolysis (Boron and Boulpael, 2012). The osteoblasts then pump the calcium into the extracellular matrix, thus contributing to mineralization. Also, PTH stimulates bone synthesis indirectly in that osteoclastic bone resorption leads to the release of growth factors such as insulin-like growth factor 1 (IGF-1), IGF 2 and transforming growth factor beta (Boron and Boupael, 2012).

3.2 Vitamin D

Vitamin D is a lipid soluble hormone. It acts like a group I (steroid) hormone, which interacts with nuclear receptors in target cells. However, it is also hydrophilic, as it contains three hydroxyl groups. Hence, it also acts like a group IIB hormone, acting through membrane receptors with cyclic guanosine monophosphate (cGMP) as the second messenger (Michael and Sircar, 2010).

Vitamin D is provided as both vitamin D2 (plant sources) and vitamin D3 (meat and fish) in the diet. In addition, vitamin D3 can be formed by the action of light on pro-vitamin D3 (7-dehydrocholesterol) present on the skin. Endogenous synthesis of vitamin D3 is important since deficiency may arise through inadequate exposure to sunlight or inadequate oral intake (Glasby and Huang, 1995). Vitamin D2 and D3 are of identical potency in humans, and are collectively referred to as cholecalciferol (Moffett et al, 1993).

Cholecalciferol is transported to the liver bound to a specific α-globulin (vitamin D transport protein), where hydroxylation at the 25 position in the liver produces 25-hydroxycholecalciferol (25-HCC; calcidiol). The 25-hydroxycholecalciferol is then further converted in the cells of the proximal tubules of the kidneys by the action of 1-hydroxylase to the more active metabolite, 1, 25-dihydroxycholecalciferol (1, 25-DHCC), which is also called calcitriol [6]. The kidneys also contains 24-hydroxylase, which can convert 25-HCC to 24, 25 dihydroxycholecalciferol (24, 25-DHCC) and a 26 hydroxylase which coverts 25-HCC to 25, 26 dihydroxycholecalciferol (25, 26-DHCC), although the significance of these other metabolites are unknown. Calcitriol is also produced in the placenta, in keratinocytes in the skin and in macrophages (Barrett et al, 2012).

1, 25-DHCC (calcitriol) mediates its biologic effects by binding to a member of the nuclear receptor superfamily, the vitamin D receptor (VDR). This receptor belongs to the subfamily that includes the thyroid hormone receptors, the retinoid receptors, and the peroxisome proliferator-activated receptors (Kasper et al, 2005). The VDR binds to target DNA sequences as a heterodimer with a retinoid X receptor, recruiting a series of coactivators that result in the induction of target gene expression (Kasper et al, 2005). The affinity of the VDR for calcitriol is approximately three times of magnitude higher than for the other vitamin D metabolites. Under normal physiologic

circumstances, these other metabolites do not appear to stimulate receptor-dependent actions (Kasper et al, 2005).

Vitamin D has multiple systemic effects on target organs **(Figure 15)**. Vitamin D promotes intestinal absorption of calcium and phosphate, which contributes to increased bone mineralization. It serves its function by induction of synthesis of calcium channels, pumps and calcium binding proteins intracellularly. Calcium moves from the intestinal lumen to the blood by both paracellular and transcellular routes. In the paracellular route, which occurs throughout the small intestine, calcium moves passively from the lumen to the blood, which is not regulated by calcitriol (Boron and Boulpael, 2012). The transcellular route, which occurs only in the duodenum, involves three steps: (1) calcium enters the cell across the apical membrane by calcium channels and endocytosis (2) calcium binds to several high affinity bindings proteins intracellularly, most prominently calbindin (3) the enterocyte extrudes calcium across the basolateral membrane by means of both a calcium pump and an Na-Ca exchanger (Boron and Boulpael, 2012). Calcitriol also appears to stimulate intestinal phosphate uptake via genomic upregulation of Na-phosphate symporter (Boron and Boulpael, 2012).

In the kidneys, calcitriol appears to stimulate reabsorption of calcium via increased TRPV5 expression in the distal convoluted tubules, with its effect

significantly enhanced with the synergistic effect with PTH (Barrett et al, 2012). As with enterocytes, calbindin appears to be present in renal tubular cells and probably has a role in increasing calcium absorption (Michael and Sircar, 2010). Additionally, albeit a lesser role than PTH, calcitriol also appears to promote phosphate reabsorption in the proximal tubule.

Figure 15. Vitamin D metabolism and physiological effects at target organs (Raff and Levitzky, 2011)

The actions of vitamin D on bone are more complex, in that it promotes both bone deposition and bone resorption. This is due to both osteoblasts and osteoclast precursor cells holding VDRs. Additionally, activated osteoblasts secrete osteoclast stimulating factor, which induces the development of osteoclasts from their precursors (Michael and Sircar, 2010). Indirectly, vitamin D provides for increased extracellular calcium from the intestine and kidneys to provide for mineralization of previously un-mineralized osteoid. However, the direct effect of vitamin D on bone is thought to mobilize calcium out of bone (Boron and Boulpael, 2012). In response to vitamin D, osteoblasts produce a number of proteins, including alkaline phosphatase, collagenase and plasminogen activator (Boron and Boulpael, 2012). However, vitamin D, like PTH also promotes the development of osteoclasts from precursor cells, thus the net effect in vitamin D in experiments using vitamin D deficient animals appears to mobilize calcium from bone into the

extracellular medium (Boron and Boulpael, 2012, Kacsoh, 2000). At the same time, the relative hypercalcaemia appeared to promote mineralization of previously un-mineralized osteoid- at the expense of bone resorption from other sites (Boron and Boulpael, 2012).

Finally, vitamin D is able to provide direct negative feedback back to the parathyroid glands to control rises in serum calcium. It does so via the 5'regulatory region of the PTH gene within chief cells of parathyroid glands, which has a VDR sequence (Boron and Boulpael, 2012). When vitamin D is occupied by the VDR complex, this element is able to reduce transcription of PTH product.

3.3 Calcitonin

Calcitonin is a small peptide hormone with a molecular weight of 3500, containing 32 amino acids produced by "clear" or C cells of the thyroid gland. They are derived from the neural crest cells of the fifth branchial pouch, which in humans, migrate into the evolving thyroid gland (Glasby and Huang, 1995). Calcitonin is synthesized in the secretory pathway by post-translational processing of a large pro-calcitonin (Boron and Boulpael, 2012). It is stored in secretory vesicles within the C cells, whereby its exocytosis is stimulated by a rise of extracellular calcium above normal, and conversely lowering the extracellular calcium diminishes its secretion. B adrenergic agonists, dopamine, estrogens, gastrin, cholecystokinin, glucagon

and secretin have also been implicated in stimulating calcitonin secretion, with gastrin being the most potent stimulus (Barrett et al, 2012). Thus, the plasma calcitonin is seen to be elevated in Zollinger-Ellison syndrome and in pernicious anaemia (Barrett et al, 2012). However, neither surgical or radio-thyroidectomy, (which eliminates calcitonin from the circulation), nor does rare calcitonin secreting tumour of the C cells, with frequent plasma calcitonin concentrations that are 50-100 times above normal significantly alters plasma calcium levels. This probably indicates that calcitonin probably does not play a major role in the physiologic regulation of plasma calcium in humans (Kacsoh, 2000). In any event, the actions of calcitonin are short lived, because it has a half-life of less than 10 minutes (Barrett et al, 2012).

The cellular effects of calcitonin are mediated through G protein coupled receptors from the same receptor family as PTH and PTHrP receptor superfamily (Raff and Levitzky, 2011). They are found mainly in bone and kidneys. Within bone, the osteoclast, which lacks PTH receptors but with the presence of calcitonin receptors, appears to inhibit the resorptive activity of the osteoclast and slows the rate of bone turnover (Boron and Boupael, 2012). In patients who are in the osteolytic stage of Paget's disease, but not in healthy adults, the acute inhibition of osteoclastic activity by parenteral administration of calcitonin was accompanied by a decline in plasma concentration of calcium (Kacsoh, 2000). However, exogenous calcitonin lost its effectiveness, perhaps due to receptor downregulation (Kacsoh,

2000). In the kidneys, calcitonin causes a mild phosphaturia by inhibition of proximal tubule phosphate transport, mild natriuresis and calciuresis. However, these renal effects are believed to be of short duration, and do not appear to be important in the overall renal handling of calcium, phosphate or sodium (Boron and Boulpael, 2012). Calcitonin has been a useful pharmacologic agent to suppress bone resorption in Paget's disease and osteoporosis, and in the treatment of hypercalcaemia of malignancy.

3.4 Parathyroid Hormone Related Protein

The paracrine factor Parathyroid Hormone Related Protein (PTHrP) is responsible for most instances of hypercalaemia of malignancy, a syndrome that resembles hyperparathyroidism (Kasper et al, 2005). It is manufactured in a wide variety of normal tissues, including brain, pancreas, heart, lung, mammary tissue, placenta, endothelial cells and smooth muscles. It is also commonly implicated in squamous cell cancers of lung, head and neck, renal and bladder carcinomas. PTHrP is a product of a single multi-exonic gene, which is organised similar to that of PTH, however its genes are completely separate to that from PTH. Due to alternative splicing, three translation products are synthesized, and eventually secreted (Kacsoh, 2000):

- PTHrP (1-36) is homologous with PTH and acts on PTH/PTHrP receptor and N terminal PTHrP-specific receptor;

- Mid-region PTHrP [PTHrP (38-94), (94-95) and (38-101)] acts on its own unique receptor;
- C-terminal PTHrP (107-139), termed osteostatin for its direct osteoclast inhibiting action, which involves a distinct receptor.

The similar actions of PTHrP and PTH arise from homology within the first 13 amino acids of PTHrP (as above) with native PTH, with only weak homology seen between amino acids 14-34 (3 amino acids are identical), and essentially no homology beyond amino acid 34 (Boron and Boulpael, 2012).

The normal physiologic functions of PTHrP are still largely unknown. In non lactating humans, the plasma PTHrP concentration is kept at very low levels, and does not appear to be involved in day to day regulation of serum calcium levels. It is thought to contribute to local stimulation of calcium transport, activation of renal 1α-hydroxylase activity in the kidney, and provide local hormonal response in bone turnover and calcium homeostasis (Kasper et al, 2005). PTHrP is seen to exert important development influences on foetal bone development and in adult physiology. A homozygous knockout of the PTHrP gene in mice causes a lethal deformity in which animals are born with severe skeletal deformities resembling chondrodysplasia [8]. Additionally, the lactating breast in humans secretes PTHrP, and this hormone is present in very high concentrations in

milk (Boron and Boulpael, 2012). PTHrP may promote the mobilization of calcium from maternal bone during milk production (Boron and Boulpael, 2012).

3.5 Potassium

There appears to be an association between potassium and calcium/bone homeostasis through observational studies and randomized controlled trials. The most promoted hypothesis for a bone benefit of potassium is through its effect on acid-base balance. There appears to be a complex interaction between the interstitial fluid that surrounds the crystalline bone, and the systemic extracellular fluid. The bone's interstitial fluid has higher concentrations of potassium and sodium, and lower concentrations of calcium and phosphorous, compared with plasma (Tylavsky et al, 2008). The potassium content of the bones' interstitial fluid appears to be directly related to the quantity of potassium consumed, and is the skeletal compartment's first line of defense in buffering metabolic acid loads (Tylavsky et al, 2008; Green et al, 1991).

Alkaline potassium salts produced from metabolizing fruits and vegetables and potassium supplements appear to protect against bone resorption from low grade metabolic acidosis from western diets or other insults, by neutralizing the acid (Wever et al, 2013). In addition, potassium intake has

been associated with reduced urinary calcium excretion, suggesting that potassium may have a role beyond its alkalization effect (Dawson-Hughes B et al, 2009). The positive effect of potassium on bone could be through either the suppression of calcium resorption, bone mineral dissolution, or both (Wever et al, 2013). Studies by Sebastian et al in post-menopausal women receiving potassium suggested that this improved calcium balance, and reduced net renal acid excretion. Further, a trial by Jehle et al demonstrated that potassium citrate given at 50mmol/day over 2 years improved bone mineral density and bone microarchitecture in 201 elderly men and women (Jehle et al, 2013).

3.6 Gonadal steroid hormones

Gonadal hormones (oestrogen and testosterone) are not directly involved in regulation of plasma calcium, but they contribute to the overall long term maintenance of normal bone mass after puberty. In the absence of gonadal steroids, calcium is steadily lost from bones, leading to osteoporosis (Moffett et al, 1993). In males, levels of testosterone are adequately maintained to reduce the chance of osteoporosis despite a fall in secretion of testosterone, however in females, oestrogen levels fall below the level that maintains bone mass, particularly after menstruation, or if reproductive cycles are interrupted by an eating disorder or intensive athletic training (Moffett et al, 1993).

Chapter 4: Pathophysiology of parathyroid glands in chronic (end stage) renal failure

The kidney is essential for maintaining the homeostasis of divalent ions and the metabolism of vitamin D. They act to maintain the balance for calcium, phosphorus and magnesium and they are the principal tissues responsible for hydroxylating 25-hydroxyvitamin D to 1, 25-dihydroxyvitamin D and 24, 25-dihydroxyvitamin D. With the progressive chronic reduction in the glomerular filtration rate, renal hyperparathyroidism ensues. About 10-30% of patients with chronic renal failure undergo parathyroidectomy to control secondary and tertiary hyperparathyroidism. Secondary hyperparathyroidism is defined as excess PTH secretion from parathyroid glands from a persistent hypocalcaemic stimulus arising from end stage renal failure. Tertiary hyperparathyroidism is defined by either (a) Excess PTH secretion resulting in hypercalcaemia in end stage renal failure or (b) autologous excess PTH secretion, despite corrective measures with renal transplant. This percentage has remained constant over the last twenty years despite advances in medical therapy (Parfrey et al, 2013). The pathophysiology of this disorder is complex and involves a number of feedback loops between the kidneys, bone, intestine and the vasculature. The main goal of this system is maintenance of calcium and phosphorous balance, often at the expense of abnormalities in other components of the system (Berkoben and Quarles,

2015). Several interrelated factors are thought to be involved in the pathogenesis of refractory hyperparathyroidism, including phosphate retention, decreased free ionized calcium concentration, decreased 1,25-dihydroxyvitamin D (calcitriol) concentration, increased fibroblast growth factor 23 (FGF-23) concentration, reduced expression of vitamin D receptors (VDRs) and calcium sensing receptors (CaSRs), and hyperplasia of parathyroid glands (Jowsey et al, 1974). Renal osteodystrophy appears to be an almost universal phenomenon resulting from persistent secondary and tertiary hyperparathyroidism, which is a high state of bone turnover, leading to osteitis fibrosa, usually characterized by increased osteoclast and osteoblast activity and peri-trabecular fibrosis.

4.1 Pathophysiology of chronic renal failure-mineral bone disease- secondary hyperparathyroidism

4.1.1. Phosphate retention

Many investigators have demonstrated that phosphorous retention in the course of renal failure plays an important role in the pathogenesis of renal hyperparathyroidism (Jowsey et al, 1974, Rutherford et al, 1977, Slatopolsky et al, 1971). While it was initially proposed that phosphate retention would give rise to a decrease in the levels of ionized calcium that would stimulate PTH release, additional observations have indicated that hypocalcaemia is not necessary for hyperparathyroidism to develop and therefore point to the fact that other consequences of phosphate retention are likely to be important

(Rutherford et al, 1977). Nonetheless, it has been clearly demonstrated that if phosphate in the diet is restricted in proportion to the decrease in kidney function, hyperparathyroidism can in part, be reversed (Rutherford et al, 1977, Aly et al, 1995). The mechanism whereby phosphate retention leads to hyperparathyroidism is not certain. From the viewpoint of phosphate homeostasis, the initial elevation of PTH secretion is appropriate since the ensuing increase in phosphate excretion lowers the plasma phosphate concentration toward normal. Among patients with severely reduced eGFR, PTH inhibits proximal tubule phosphate reabsorption from the normal 80-95% to as low as 15% of the filtered phosphate (Cunningham et al, 2011, Slatopolsky et al, 1971). In addition, it is possible that phosphate retention could serve to decrease the production of calcitriol production, and the consequences of reduced calcitriol production could contribute to hyperparathyroidism. Other investigators have demonstrated that hyper-phosphataemia stimulated parathyroid gland growth, and that elevations in phosphate stimulated the secretion of PTH independent to changes in the levels of ionized calcium (Slatopolsky, 1996, Almaden et al, 1999). The stimulation of parathyroid growth by a high phosphate load appears to be associated with increased expression of transforming growth factor alpha (TGF-α), which acts on the epidermal growth factor receptor (EGF-R) to activate mitogen-activated protein (MAP) kinase and stimulate cell proliferation (Dusso et al, 2001). This effect of phosphorous appears to be

post-transcriptional and to be mediated by alterations within the parathyroid cell that affect the stability of PTH messenger RNA (Aly et al, 1995). An alteration in dietary phosphate intake has also been shown to regulate the expression of the calcium sensing receptor in the parathyroid gland and thereby influence the response of the parathyroid to changes in serum calcium (Aly et al, 1995). Overt hyperphosphataemia does not generally appear in patients with renal failure until their renal function decreases to below 25% normal. This is probably due to maintenance of normal phosphataemia as a consequence of increased levels of PTH in promoting phosphate excretion from the kidneys above this renal function level. However, with long term reduction of eGFR, since phosphate reabsorption by the renal tubules cannot be lowered down below a minimum threshold, continued PTH-induced release of phosphate form bone leads to exacerbation of hyperphosphataemia (Berkoben AND Quarles, 2015).

4.1.2. Decreased calcitriol activity

Plasma calcitriol concentrations generally fall below normal when the eGFR is <60ml/min, although low concentrations have also been found in some patients with higher eGFR <80ml/min (Levin et al, 2007). 80% of chronic renal failure patients are thought to be vitamin D deficient. Initially, the decline in calcitriol concentration is likely to be due to the increase in fibroblast growth factor-23 (FGF-23) concentration rather than the loss of functioning renal mass. The FGF-23 induced decrease (by suppressing the

activity of 1-alpha hydroxylase) in calcitriol begins early, when the eGFR drops to <70ml/min, however in advanced renal failure, hyperphosphataemia and loss of renal mass may also contribute to the decline in calcitriol synthesis (Berkoben and Quarles, 2015). Phosphate retention can directly suppress the renal synthesis of calcitriol by inhibiting 1-alpha-hydroxylase activity (Llach, 1995). Hyperphosphataemia stimulates the secretion of FGF-23 (primarily by bone osteocytes) which act on target tissues by binding onto its receptor. Thus, this provides a trigger for increased PTH production from the parathyroid glands (Berkoben and Quarles, 2015).

Inability of the diseased kidney to produce calcitriol plays an important role in the pathogenesis of altered calcium homeostasis in renal insufficiency (Coburn et al, 1976). In patients with advanced renal failure, the intestinal absorption of calcium is impaired and the plasma levels of calcium and calcitriol are uniformly depressed. Moreover, the administration of modest doses of calcitriol can restore the intestinal absorption of calcium to normal uraemic patients (Yuen et al, 2016, Liebross and Coburn, 1982).

A demonstration of vitamin D deficiency resulting in parathyroid hyperplasia and increased PTH synthesis comes from studies whereby administration of calcitriol inhibits parathyroid gland growth, prevention of the decrease in parathyroid vitamin D receptor (VDR) expression and control of PTH.

Binding of calcitriol to VDR resulted in trans-repression of the PTH gene and increased mRNA levels for VDRs, due to calcitriol induction of CEBPβ, a trans-activator of the VDR gene in renal cells and in osteoblasts (Dhawan et al, 2005, Dusso and Brown, 2009). It also prolonged the VDR half-life through ligand dependent protection of the VDR from proteosomal degradation (Wiese et al, 1992). In addition, it appears that calcitriol interaction with VDR also stimulated induction of calcium sensing receptor (CaSR) gene. In rats, parathyroid CaSR mRNA levels were decreased by 40% by vitamin D deficiency, and enhanced by calcitriol treatment in a time and dose dependent manner (Dusso and Brown, 2009). Thus, in chronic renal failure, both parathyroid levels of CaSR and VDR receptor levels decrease, which reduces the inhibitory ability of calcium and calcitriol to PTH release. Simultaneously, the parathyroid glands undergo hyperplasia, and with progressive disease, it forms nodules (nodular hyperplasia), the most severe form of secondary hyperparathyroidism, insensitive to high calcium and calcitriol suppression of either PTH or cellular growth (Dusso and Brown, 2009).

4.1.3. Skeletal resistance to the calcaemic action of parathyroid hormone

Another factor which contributes to hypocalcaemia and the degree of secondary hyperparathyroidism in renal failure is skeletal resistance to calcaemic action of PTH. Thus, a higher concentration of PTH may be required to maintain serum calcium at normal levels. In patients with

advanced renal failure and in those treated with dialysis, the administration of exogenous parathyroid extract fails to produce a normal increment rise of serum calcium (Massry et al, 1973). In addition, patients with mild renal insufficiency exhibit delayed recovery form the hypocalcaemia induced by the infusion of ethylenediamie tetracetic acid (EDTA) despite a greater than normal increment rise in serum PTH (Kraut et al, 1981). Resistance to PTH appears to be multifactorial, but is likely due to downregulation of PTH receptors induced by the high circulating PTH concentrations, calcitriol deficiency and hyperphosphatemia (Rodriguez et al, 1991). Also, PTH fragments with a truncated N-terminus, previously considered to have no biological action, may actually be involved in the regulation of skeletal metabolism (Aly et al, 1995). The actions of such N-terminally truncated PTH fragments appear to oppose the calcaemic effects of PTH; their accumulation the serum in renal failure could therefore contribute to skeletal resistance to PTH (Aly et al, 1995). One such fragment, PTH 7-84 has been shown in vitro to be a potent inhibitor of stimulated bone resorption due to PTH, calcitriol, interleukin 11 or prostaglandin E2 (Divieti et al, 2001).

4.1.4. Decreased fibroblast growth factor 23

Fibroblast growth factor 23 (FGF-23) is a circulating peptide that plays a role in the control of serum phosphate concentrations. It is secreted by bone

osteocytes and osteoblasts influenced by serum calcitriol, phosphate, PTH and calcium levels and its key function is to maintain normal phosphate concentration by reducing phosphate reabsorption and by reducing intestinal phosphate absorption through decreased calcitriol production (Qunibi et al, 2015). In the kidneys, FGF-23 binds to its receptor (FGF-R) and its co-receptor, klotho, causing inhibition of the expression of the Na/Pi cotransporter. Klotho, a transmembrane protein, is required for FGF-23 receptor activation (Urakawa et al, 2006). In addition, FGF-23 also inhibits expression of 1-alpha-hydroxylase enzyme, leading to reduced calcitriol production from the kidneys (Qunibi et al, 2015). It also appears that FGF-23 directly suppresses PTH production, however in chronic renal failure, the presence of high PTH concentrations suggests that the parathyroid gland is relatively resistant to the elevated concentrations of FGF-23 in uraemia (Qunibi et al, 2015).

Patients with chronic renal failure appear to have increased FGF-23 concentrations due to phosphate retention and decreased clearance. Klotho expression declines early and progressively in the course of renal failure, whist the concentration of FGF-23 elevates, suggesting there is a direct inverse relationship between these two. Moreover, the decrease in klotho expression on hyperplastic parathyroid glands may contribute to the resistance and impaired parathyroid suppression by FGF-23 (Qunibi et al, 2015, HU et al, 2011).

4.2 Tertiary hyperparathyroidism

In some patients with longstanding secondary hyperparathyroidism, they develop markedly elevated PTH levels associated with hypercalcaemia, unexplained by the administration of calcium or calcitriol to counteract it. Such patients are known to have tertiary hyperparathyroidism, who are often refractory to medical therapies due to the autonomous secretion of PTH that is no longer responsive to the plasma calcium concentration (Qunibi et al, 2015). Tertiary hyperparathyroidism is often seen in the context of renal transplantation (in the view to correct secondary hyperparathyroidism), whereby 15-50 per cent of transplanted patients after their first year still have hyperparathyroidism, which is unlikely to improve spontaneously (Dulfer et al, 2017). Its occurrence is thought to be related to the severity of pre-transplant hyperparathyroidism and duration of dialysis prior to transplantation (Park et al, 2011). In patients with tertiary hyperparathyroidism, reduced expression of CaSR and VDRs leads to a lack of suppression of PTH by calcium and vitamin D, which results in prolonged stimulation of parathyroid gland hyperplasia (Qunibi et al, 20150. Nodular parathyroid hyperplasia may result, which is resistant to regression despite resolution of the triggering mechanisms (such as medical therapy or after renal transplantation).

4.3 Clinical manifestations of renal hyperparathyroidism

Clinical manifestations of renal hyperparathyroidism are usually non-specific and often preceded by biochemical or imaging abnormalities.

4.3.1. Musculoskeletal symptoms

In patients with advanced renal insufficiency who have severe bone disease, bone pain is a common manifestation. This is often nonspecific in nature, and occurs in the lower back, hips, legs, and is aggravated by weight bearing (Gonzalez and Martin, 2000). Acute, localized bone pain can also become manifest and may be suggestive of acute arthritis. Pain around joints may be caused by acute peri arthritis, which is associated with periarticular deposition of calcium phosphate crystals, especially in patients who suffer from marked hyperphosphatemia (Gonzalez and Martin, 2000). The symptoms may be confused with gout or pseudo-gout.

Proximal muscle weakness is usually of gradual onset and may be severe and debilitating in some patients with advanced renal failure. Muscular weakness may be so profound that affected individuals may have difficulty getting up from a sitting position (Heptinstall, 1983). The plasma levels of muscle enzymes are normal and there are no characteristic abnormalities on electromyography (Aly et al, 1995). Proximal myopathy and muscle weakness may be related to secondary hyperparathyroidism, phosphate depletion and vitamin D deficiency. Muscle weakness may also arise as a

result of peripheral neuropathy, electrolyte disturbances, iron overload and carnitine deficiency. Spontaneous tendon rupture has been observed in patients with long-standing renal disease in dialysis (Aly et al, 1995). The quadriceps, triceps and Achilles tendons have been most commonly implicated. Involvement of the extensor tendons of the fingers has also been described. The proximal muscle weakness is to a large extent, reversed by parathyroidectomy, which abolishes the PTH elevation. How the PTH relates to the causation of this myopathic weakness remains incompletely understood.

Spontaneous fractures most commonly affect the axial skeleton where they involve the vertebral bodies, ribs and hips. Such fractures are most commonly associated with ostomalacia (low bone turnover and poor mineralization), adynamic bone disease (low turnover pathology with normal mineralization) and/or ostitis fibrosa cystica (high bone turnover), collectively known as renal osteodystrophy (Yuen et al, 2016). Fractures often occur with minimal trauma. Thus, crush vertebral fractures may occur spontaneously, rib fractures can occur during a sneeze/cough, and hip fractures may occur such as when a patient steps off a curb. Less frequently, fractures involve the long bones (Liebross and Coburn, 1982). Spontaneous rib fractures are frequently multiple, and little displacement may be seen in chest x ray, hence, they are more easily visualized with a bone scan, indistinguishable from stress fractures or pseudo fractures (Liebross and

Coburn, 1982). With longstanding bone resorption, patients may develop localized regions of bone loss that are often replaced by fibrous tissue, resulting in a brown tumour (Yuen et al, 2016). These brown tumours appear as well defined lytic lesions on x ray and may be mistaken for metastasis (**Figure 16**)

Figure 16. X ray of a 55 year old patient with renal osteodystrophy and brown tumours of the fourth metacarpal and third proximal phalanx of the left hand (arrows) (Liebross and Coburn, 1982)

4.3.2. Pruritus

This is especially common and troublesome in patients who have chronic renal failure. Substantial or total improvement may follow after parathyroidectomy. The mechanism responsible for pruritus is also not well understood, and may be related to a change in the calcium content of the skin. However, the treatment of severe pruritus is often symptomatic, but is an indication for consideration for parathyroidectomy.

4.3.3. Metastatic calcification

There are two forms of extra-skeletal or metastatic calcification (1) amorphous calcium phosphate, found in soft tissues such as heart, lung and kidney and (2) hydroxyapatite, similar to that of normally calcifying tissue present in vascular, valvular, joint and ocular tissues. Metastatic and extra-

skeletal calcifications can occur in damaged tissue (dystrophic calcification) or in apparently normal tissue (Aly et al, 1995). This calcification occurs in a variety of tissues both visceral and non-visceral, including skin, cartilage, heart, lungs, kidneys, and shoulders, limbo-conjunctival, vascular and valvular tissues (Aly et al, 1995). Calcification of the cardiovascular tissue can affect the myocardium, atrial-ventricular conduction, and valvular function (Liebross and Coburn, 1982). This has significantly received attention as they have been linked to increased risk of cardiovascular events and death in chronic renal failure (Aly et al, 1995, Wang et al, 2003).

4.3.4. Calciphylaxis

Calciphylaxis is an unusual yet devastating syndrome characterized by skin, fat, digit and limb necrosis that is attributed to medial calcification of small and medium sized arteries. This condition has been described in patients with end stage renal failure, particularly on long term dialysis and it usually occurs in the setting of uncontrolled hyperparathyroidism. The skin lesions initially manifest as painful nodules with *peau d'orange* changes which may become mottled with violaceous discolouration similar to livedo reticularis, and can subsequently become infected. The lesions can be found in the distal extremities, involving the toes, fingers or ankles, or they may be localized in proximal areas such as thigh, buttocks, abdominal wall or breasts (Aly et al, 1995). Histologic examination of the involved skin demonstrates medial calcification of small and medium sized vessels. The pathogenesis of this

lesion is obscure, however since these lesions are similar to those seen with warfarin induced skin necrosis, a role for altered coagulation, particularly in the protein C and S pathway in the final manifestations of this issue has been considered. Patients on dialysis with calciphylaxis have been noted to have decreased protein C and protein S activity (Kant et al, 1992). On the whole, calciphylaxis presents as a serious complication, since disseminated infection from tissue necrosis is the most common cause of death. In general, calciphylaxis carries a very poor prognosis, even after parathyroidectomy (Gonzalez and Martin, 2000).

Chapter 5: Investigations and preoperative localisation of parathyroid glands

5.1 Investigations

These are planned in the first instance to establish whether there is hypercalcaemia, to assess the extent of hyperparathyroidism and gauge the severity of renal osteodystrophy. For dialysis patients, the suggested *target goals* are (Quarles and Berkoben, 2015):

- Serum levels of corrected calcium levels should be maintained between 2.10-2.37mmol/L;
- Serum levels of phosphate should be maintained between 1.13-1.78mmol/L;
- Second generation PTH assay should be maintained between 2.5 to 5 times the upper limit of normal.

5.1.1. Plasma calcium

Although ionized calcium is the physiologically active component regulating PTH secretion, its measurement presents technical difficulties and is not routinely measured. Generally, the sum of ionized, protein bound and complexed forms is measured (referred to plasma calcium), however current assays can provide plasma calcium levels minus the bound albumin component (referred to plasma corrected calcium) (Toft et al, 1981).

Changes in H+ concentration may affect plasma calcium levels by altering the distribution between ionized and protein-bound forms, independent of a disturbance of calcium homeostasis (Toft et al, 1981). The average serum calcium level of patients with advanced renal failure is lower than that measured in a control population, however dialysis dependent chronic renal failure patients often have hypercalcaemia due to secondary or tertiary hyperparathyroidism. It may be associated with excess intake of oral calcium and treatment with various vitamin D sterols, and it is not uncommon in patients with the syndrome of 'dialysis osteomalacia' (Heath and Marx, 1982). Changes in plasma calcium in response to dairy intake are small, but it is preferable to collect specimens under fasting conditions.

5.1.2. Serum phosphate

Serum phosphate generally is normal in early renal failure, however as the eGFR falls to less than 25% of normal, patients become hyperphosphataemic. At this degree of renal failure, changes in dietary phosphate intake can greatly contribute to the degree of serum phosphate levels (Heath and Marx, 1982). In addition, it is usually necessary to treat patients with oral phosphate binders. Treatment with calcitriol can also stimulate the absorption of both phosphate and calcium from the intestine, and thus may aggravate hyperphosphataemia (Heath and Marx, 1982). It may be intuitive to think that hyperphosphataemia stimulates the production of PTH from parathyroid glands to provide a phosphaturic action,

however at eGFR levels <10ml/min, this physiological counteraction appears de-functional secondary to end stage renal failure.

5.1.3. Parathyroid Hormone

PTH levels are a direct measure of parathyroid gland function, and an indirect measure of bone remodelling. Full length PTH has a half-life of 2-4 minutes, which it is cleaved into an inactive C-terminal fragment, an active N-terminal fragment, and an inactive mid-region fragment in the peripheral tissues (Gilbert et al, 2014). A two-site immunoassay is currently used to measure circulating PTH concentrations, utilizing two antibodies. One antibody detects an epitope near the N-terminus, and the other detects the C-terminal end. The assay actually detects the full length bioactive PTH (1-84) and PTH fragments such as PTH (7-84), albeit the non-biologic fragment (Gilbert et al, 2014). Newer, second generation immuno-active PTH assays are also available that recognize amino acids 1-4 of the N-terminus, and specifically detect full length PTH (1-84) (Gilbert et al, 2014). PTH levels greater than 31.50pmol/L generally correlate with bony changes of secondary hyperparathyroidism and/or osteitis fibrosis. PTH is also a crude, indirect measure of bone turnover, however alkaline phosphatase levels can be used in conjunction to determine the degree of osteoblastic activity (Gilbert et al, 2014).

5.1.4. Alkaline phosphatase

The total serum alkaline phosphatase reflects enzyme activities arising from hepatic, intestinal and skeletal sources. Despite the non-specific nature of alkaline phosphatase, a slow progressive increase in serum level in end stage renal failure may be the only clue to the development of overt bone disease (Heath and Marx, 1982). The skeletal alkaline phosphatase arises largely from osteoblasts, and a correlation between skeletal alkaline phosphatase and bone biopsy findings of osteitis fibrosa has been observed (Heath and Marx, 1982, Pierides et al, 1979).

5.1.5. Serum magnesium

The levels of serum magnesium tends to increase as the eGFR falls below 20-25% of normal. Acute hypermagnesemia may occur in end stage renal failure patients following an increase in dietary magnesium intake and those ingesting magnesium containing antacids (Heath and Marx, 1982).

5.1.6. Radiology

Radiographic studies are generally not required in the diagnosis of bone disorders secondary to renal hyperparathyroidism, although certain radiographic changes can be seen. Sub periosteal erosions of the phalanges and of the terminal digits tufts can often be seen on hand x ray, and these changes may also be present in the outer third of clavicle, both the neck and distal end of the femur and the upper end of the tibia (Toft et al,

1981). Expansile lytic lesions (brown tumours), as well as cysts which may be solitary or multiple in long bones may be seen in severe osteitis fibrosis cystica **(Figure 14)**.

There is no accurate correlation between bone mineral density (BMD) as measured by dual energy X ray absorptiometry (DEXA) and the type of chronic renal failure associated bone disease present (Gilbert et al, 2014). Although patients with chronic renal failure typically have lower BMDs than the general population, the interpretations of DEXA scans are further complicated in secondary hyperparathyroidism because of focal areas of osteosclerosis, the presence of extra-skeletal calcifications and the variable presence of osteomalacia (Gilbert et al, 2014). However, analysis of BMD at distal sites, such as the distal radius, or the hip may be useful in assessing fracture risk in chronic renal failure. BMD may also be considered in patients who have undergone renal transplantation, or who have known risk factors or previous fractures and are candidates for osteoporosis therapy (Gilbert et al, 2014).

5.2 Preoperative localisation of parathyroid glands

The diagnosis of hyperparathyroidism is established by the patient's symptoms and biochemical data, and is not influenced by a positive or negative result of any parathyroid imaging modality. A single focus positive imaging result does not reliably exclude the presence of multi-glandular

parathyroid disease (Yip et al, 2015). Likewise, medical or surgical management decisions are made according to the severity of the disease with the goal of parathyroidectomy the restoration of normo-calcaemia and it should not be postponed if a parathyroid abnormality is not portrayed in imaging tests (Moralidis, 2013).

However, unlike the well-studied role of localization studies in patients with primary hyperparathyroidism, little is known about the usefulness in patients with secondary and tertiary hyperparathyroidism. Most of the published literature is in the form of small case series. Considering that the majority of patients with renal hyperparathyroidism will require a bilateral neck exploration, and the fact that the parathyroid glands are larger than seen in primary hyperparathyroidism, the utility of localizing studies is unproven (Alkhalili et al, 2015). Additionally, the sensitivity of imaging studies has been reported to be lower than in primary hyperparathyroidism, where the preoperative use of combined neck ultrasound, parathyroid scintigraphy and/or four dimensional computed tomography (4D-CT) has recently been proposed for minimally invasive explorations (Fuster et al, 2006, Lumachi et al, 2001). On the other hand, the relatively small size of some hyperplastic or adenomatous glands and the common occurrence of unexpected ectopic and/or supernumerary glands have led to a relatively high rate of reoperation due to missed glands located in the neck (Fuster et al, 2006). This also raises the question of the need to perform preoperative

imaging in order to increase the success rate of surgery in renal hyperparathyroidism (Fuster et al, 2006).

In light of poor sensitivity, most surgeons do not opt for any preoperative imaging, and believe that the best localizing tool is to 'localize an experienced parathyroid surgeon'. However, if imaging modalities are employed, the most commonly used are ultrasound, parathyroid scintigraphy, CT and/or MRI.

5.2.1. Ultrasound

Neck ultrasonography is often utilized for localisation **(Figure 17)**. However, the accuracy of ultrasound is invariably operator dependent. In one prospective study, the sensitivity and specificity of detecting hyperplastic parathyroids in uraemic hyperparathyroidism was reported as 62% and 95% respectively, and had a 0% detection rate of ectopic glands (Vulpio et al, 2010). In another study, the sensitivity and specificity of sonography for detection of secondary hyperparathyroidism were 62.5% and 85.7%. It also concluded that high resolution US provided significant correlation between enlarged parathyroid glands and parathyroid hormone level (Anari et al, 2011).

Ultrasound based scores have also been reported to be useful as predictors for nodular hyperplasia in patients with secondary hyperparathyroidism (Gwiasda et al, 2017). Studies by Gwisda et

al 2017 was able to use characteristics of blood flow patterns and echo-structure of hyperplastic parathyroid glands to identify nodular hyperplasia, indicating the need for surgical or ablative treatment when failing conservative therapy.

As with radio-nucleotide based techniques, the sensitivity of ultrasound for parathyroid localization is reduced in patients with concurrent thyroid nodules. However, ultrasound is helpful for the characterization and evaluation of any thyroid pathology, facilitating operative planning (Yip et al, 2015). This is a common problem, since concurrent thyroid pathology is present in 20-30% of patients with hyperparathyroidism (Bentrem et al, 2002). In addition, ultrasound directed fine needle aspiration with analysis of PTH levels can be useful for confirming suspected parathyroid lesions such as intra-thyroidal glands (Yip et al, 2015).

Figure 17. (a) Initial (left) and delayed (right) phase of the 99mTc-MIBI study demonstrating fixed uptake of right inferior, left superior and inferior enlarged parathyroid glands. (b) Ultrasound of neck corresponding to left inferior (left) and left superior (right) parathyroid glands, measuring 3.9x3.3mm and 3.4x3.3mm respectively (Fuster et al, 2006)

5.2.2. Parathyroid scintigraphy

Generally, 99mTc-sestamibi scintigraphy (MIBI) alone provides poor yield in localizing hyperplastic parathyroid glands. Vulpio et al 2010 reported sensitivities lower than ultrasound scans (55% vs 62%). A similar result was reported in Lee et al 2016 where they reported sensitivities as low as 56.1%. Often, ultrasound and MIBI scans are combined to improve sensitivity, with reported sensitivities of up to 73% (Vulpio et al, 2010) **(Figure 17)**.

Most centres have adopted sestamibi-single photon emission computed tomography (SPECT), a three dimensional scan that provides higher resolution imaging and improves performance of sestamibi (MIBI) scanning (Yip et al, 2015). SPECT is often combined with CT as SPECT/CT **(Figure 19)**. A recent study of sixty patients with secondary hyperparathyroidism demonstrated that SPECT/CT sensitivity, specificity and accuracy was 85%, 58% and 89%, significantly higher than compared with ultrasound alone (Kawata et al, 2009). When combined with SPECT/CT and ultrasound together, this improved sensitivity, specificity and accuracy to 93%, 61% and 97% (Kawata et al, 2009).

As recurrent disease (from ectopically placed glands) is more frequent in patients with renal hyperparathyroidism, preoperative MIBI imaging has been advocated to detect ectopic or supernumerary glands. However, the diagnostic yield of this modality is no more than moderate in eutopic gland

hyperplasia; whereas many surgeons assert that the identified aberrant glands are usually located in sites that are routinely explored during surgery (Moralidis, 2013). A recent study reported that both ultrasound and scintigraphy failed to identify 61.5% ectopic glands, and scintigraphy was able to identify 69.7% of all ectopic glands located in the mediastinal and thymic regions (de Andrade et al, 2014). However, in postoperative recurrence, scintigraphy is superior to other imaging techniques in the detection of abnormal foci, and particularly advantageous is the use of SPECT/CT (Moralidis, 2013). PET/CT or MRI may be used if previous investigations have been negative, or more invasive modalities such as selective venous sampling are justified when imaging tests are fruitless (Moralidis, 2013).

5.2.3. Four Dimensional Computed Tomography (4D-CT)

Increasingly, 4D-CT is being used as an adjunct for preoperative localization of parathyroid glands. This technique combines standard multi-planar CT scanning with a fourth dimension consisting of changes in contrast attenuation over time. This involves perfusion information derived from non-contrast, arterial, post-contrast and delayed phases (Ellika et al, 2013) **(Figure 18)**. Parathyroid glands and adenomas receive contrast enhanced blood flow a few seconds earlier than the thyroid tissue, combined with a slightly higher flow, which allow parathyroid adenomas to “lighten up” earlier than thyroid tissue, and also washes the contrast out earlier

(Lundstroem et al, 2016). As a result, 4D-CT provides both functional and anatomic information about the abnormal gland, whist permitting unbiased detection of ectopic or multi-glandular disease that may be missed by other imaging techniques (Bann et al, 2015). The first results of this modality was published by Rodgers et al in 2006, who demonstrated significantly greater sensitivity (88%) than sestamibi imaging (65%) and ultrasonography (57%) for localization of hyper-functioning parathyroid glands (Rogers et al, 2006). In an Australian study of 99 patients who had adenomas, the overall sensitivity of 4D-CT was 92% compared to 70% for sestamibi (Brown et al, 2014). The sensitivity of 4D-CT was 76% in those patients who had a negative sestamibi, and 91% for the re-operative cases (46% for sestamibi) (Brown et al, 2014). Up to 62% of those patients with negative sestamibi scans potentially avoided a bilateral neck exploration following a positive 4D-CT (Brown et al, 2014). In a survey of 361 radiologists conducted by Hoang et al, 2016, 55% of the radiologists utilized 4D-CT in their practice for localization of parathyroid adenomas. 10% used 4D-CT as the first line imaging study, 76% reported that 4D-CT played a secondary role, and 13% performed routinely in combination with ultrasound and scintigraphy (Hoang et al, 2016).

However, the major concern with 4D-CT is the radiation dosage delivered to the patient. In a study by Hoang et al, 2014, it was reported that the effective

dose (ED) of 4D-CT was more than double that of scintigraphy (28mSv vs 12mSv). Organs receiving the highest radiation dose were the thyroid (150.6mGy) and salivary glands (137.8mGy). For scintigraphy, the highest organ doses were to the colon (41.5mGy), gallbladder (39.8mGy) and kidneys (32.3mGy). The risk of carcinogenesis is of particular relevance in the younger patient group.

To this date, studies and use of 4D-CT have been largely confined to parathyroid adenomas from primary hyperparathyroidism. There currently no reports of its utility in preoperative localization of parathyroid hyperplasia in renal hyperparathyroidism.

Figure 18. 4D-CT images of non-contrast (A), arterial phase (B) and delayed phase (C). (D) demonstrates a time-attenuation curve demonstrating rapid contrast enhancement and washout from the adenomatous gland. Yellow arrow indicates a left parathyroid gland (Bann et al, 2015).

5.2.4. Magnetic resonance imaging (MRI)

MRI is seldom used in preoperative localization of the parathyroids, as cervical lymph nodes have similar imaging characteristics, which limit the accuracy of it (Yip et al, 2015). For re-operative surgery, MRI may provide a useful non-invasive imaging modality, with reported sensitivities for

abnormal parathyroid tissue from 40-85% (Wakamatsu et al, 2003, Yip et al, 2015).

5.2.5. Positron emission tomography (PET)

This modality uses 11C-methionine as a radiotracer to identify pathologic parathyroid glands **(Figure 17)**. In a study by Tang et al 2008, the use of 11C-methionine PET in preoperative localization of pathological parathyroid tissue had been shown to be as reliable as MIBI-SPECT, with its clinical benefit in providing metabolic and morphological information provided for surgical planning, reporting sensitivity of 68% in detecting parathyroid hyperplasia (Tang et al, 2008). The same authors identified that combining 11C-methionine PET/CT increased the confidence of the surgeon in identifying ectopic and supernumerary parathyroid glands, as well as when it was in close contact with surrounding structures such as the trachea, the oesophagus or cervical bone structures (Tang et al, 2008).

Figure 19. From left to right: 11C-methionine PET trans-axial slice, corresponding CT, fused 11C-methionine PET/CT and MIBI-SPECT images of a patient with secondary hyperparathyroidism with three pathological parathyroid glands (Tang et al, 2008)

5.2.6. Selective venous sampling

This is the most common invasive modality used for parathyroid localization. A 1.5-2 fold increase in PTH levels obtained from representative cervical or mediastinal vein drainage location compared to a peripheral location is considered an abnormal elevation (Yip et al, 2015). It is used when all other imaging modalities are negative. In one report of 21 patients, selective venous sampling provided localisation sensitivities of at least 90%, with no false positive results Seehofer et al, 2004).

In summary, investigations prior to parathyroidectomy for renal hyperparathyroidism are performed to confirm the presence of the disease, degree of elevation of the PTH levels, whether the serum calcium is normal or mildly elevated, serum electrolyte post dialysis (usually 1-2 days prior to surgery), and assessment of patients' fitness for surgery for anaesthesia. Localization of parathyroid glands through imaging is not always performed, unless there is evidence of recurrent hyperparathyroidism following an initial incomplete removal of all parathyroid tissue(s). This certainly is the standard practice in our institution.

Chapter 6:

Management of renal hyperparathyroidism

Renal hyperparathyroidism and the associated changes in mineral and bone metabolism are among the most challenging complications in patients with chronic renal failure, particularly in the most advanced stages of end stage renal failure. High phosphate levels, low/normal/high calcium concentrations and deficiency in vitamin D play the fundamental role in the pathogenesis of the disease (Konturek et al, 2016). In addition, recent studies have identified fibroblast growth factor 23 (FGF-23), a bone derived phosphaturic hormone as another important factor in the pathogenesis (Shimada et al, 2004).

6.1. Medical Management

The initial and predominant management in end stage renal failure in dialysis dependant patients is medical therapy. This principally involves the use of the combination of phosphate binders, vitamin D or active vitamin D analogues, and more recently, use of calcimimetics by the nephrologists. No single pharmacologic intervention is usually completely sufficient to restore disordered calcium and phosphate homeostasis. However, despite continuous and intensive medical management to control secondary hyperparathyroidism, approximately 15% of patients ultimately

require parathyroidectomy after 10 years and 38% of patients after 20 years of ongoing dialysis (Foley et al, 2005).

6.2. Surgical Management

Parathyroidectomy is an effective therapy for patients with persistent hyperparathyroidism after renal transplantation. Recent research by Komaba et al in 4428 maintenance haemodialysis patients who had undergone parathyroidectomy showed that successful surgery may reduce the risk for all cause and cardiovascular mortality in haemodialysis patients with severe, uncontrolled secondary hyperparathyroidism (Komaba et al, 2015). However, a significant proportion of patients after parathyroid surgery develop recurrent renal hyperparathyroidism ranging between 10-30% (Xu et al, 2016). The three common surgical techniques employed in parathyroid surgery include subtotal parathyroidectomy, total parathyroidectomy with auto transplantation, and total parathyroidectomy without auto transplantation. The most optimal surgical technique is still debatable; however the choice is often surgeon/institution dependant, as well as if the patient has an existing or planned renal transplant. For patients who refuse or cannot tolerate surgical options, percutaneous ethanol injection therapy, and percutaneous vitamin D injection therapy have occasionally been performed in Japan, however these interventions have not been performed as often due to the introduction of cinacalcet.

6.2.1. Indications for parathyroidectomy

Although medical therapies, including calcimimetics (e.g. cinacalcet) have allowed some patients with end stage renal failure to be treated non-operatively, symptomatic patients with markedly elevated parathyroid hormone levels should be referred for parathyroid surgery if they become refractory to medical therapy (Milas, 2016).

6.2.2. Symptomatic patients

Parathyroidectomy is indicated in those patients who have severe hyperparathyroidism (PTH>85-140pmol/L) that is refractory to medical therapies and is accompanied by:

- Severe hypercalcaemia;
- Progressive and debilitating hyper-parathyroid bone disease (renal osteodystrophy);
- Refractory pruritus;
- Progressive extra-skeletal calcification or calciphylaxis;
- Otherwise unexplained myopathy (in particular, proximal muscle weakness).

Parathyroidectomy has been shown to reduce cardiovascular calcification and to have a positive impact on blood pressure, anaemia and serum lipids (Ivarsson et al, 2015). Improvements in bone density and bone pain

also has been reported and been shown to improve long term survival of dialysis patients (Ivarsson et al, 2015).

6.2.3. Asymptomatic patients

It remains unresolved whether parathyroidectomy offers any benefit in asymptomatic individuals, although truly asymptomatic patients are rare in renal hyperparathyroidism. The PTH level at which surgery is indicated in this group is unknown, as the 2009 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines did not specify a PTH cut-off (Kdigo, 2015). In addition, there have been no randomized controlled trials to date that have compared the long term effects of medical versus surgical therapy of advanced secondary hyperparathyroidism (Berkoben and Quarles, 2016). However, a reason to consider parathyroidectomy in relatively asymptomatic individuals with elevated PTH include the realistic prospect of reducing cardiovascular disease, better blood pressure control, and reduced need for medications for hyperparathyroidism and hyperphosphatemia (Berkoben and Quarles, 2016). Parathyroidectomy may also improve bone density and reduce the risk of fracture. Multiple case series have reported increased bone mineral density after parathyroidectomy (Abdelhadi, 1998, Chou et al, 2001). One retrospective case controlled study found that parathyroidectomy was associated with reduction in hip fractures (0.68, 95% CI 0.54-0.86) and all fractures (0.69, 95% CI 0.57-0.84) (Rudser et al, 2007).

6.2.4. Patients awaiting transplantation

Consultation with the transplant unit regarding timing of parathyroidectomy is advised. There exists variability among transplant units in regards to recommended approach for potential transplant recipients who have refractory hyperparathyroidism. Most experts suggest parathyroidectomy for secondary hyperparathyroidism with moderate to severe symptoms, particularly if a recipient is not imminent. However, transplant nephrologists have differing recommendations for patients with mild or no symptoms (Berkoben and Quarles, 2016).

The rationale for not performing parathyroidectomy prior to renal transplant is that hyperparathyroidism is expected to resolve after transplantation; however some patients who have severe hyperparathyroidism prior to transplantation have persistent tertiary hyperparathyroidism associated with hypercalcaemia after transplantation, up to 50% after 12 months post-transplant (Berkoben and Quarles, 2016). Persistent hyperparathyroidism and hypercalcaemia have been associated with reduced graft function (Messa et al, 2011). On the other hand, parathyroidectomy may be safer if performed prior to transplantation, as parathyroidectomy performed after transplantation has been associated with abrupt deterioration of renal allograft function (Rostaing et al, 1997). This phenomenon has also been observed in patients with end stage renal disease with residual renal function (Tzanakis et al,

2000). To this date, it is still unknown whether long term graft function is negatively affected by parathyroidectomy (Berkoben and Quarles, 2016).

6.2.5. Persistent hyperparathyroidism after renal transplantation

Persistent hyperparathyroidism is reported to occur in approximately 15-50% of patients following transplantation, despite removal of the initial stimuli for hyperparathyroidism (Borchhardt et al, 2007). There exists large variability between institutions regarding treatment of tertiary hyperparathyroidism. Parathyroidectomy is the preferred treatment of patients with severe hypercalcaemia and/or hypophosphatemia caused by elevations of PTH (Yarlagadda et al, 2017). In a multicentre, open-label randomized study, subtotal parathyroidectomy was more likely to result in normo-calcaemia at 12 months compared with medical management with cinacalcet (100% vs 60% respectively) (Cruzado et al, 2016). Even in the absence of symptoms, many nephrologists refer for parathyroidectomy for patients with marked hypercalcaemia that is present for more than six months from time of transplantation. Some clinicians manage mild hypercalcaemia and hyperparathyroidism (PTH that is two to three times upper limit of normal) with cinacalcet initially to prevent complications of hypercalcaemia, however some nephrologists do not use cinacalcet in any transplant recipients and prefer to refer for parathyroidectomy (Yarlagadda et al, 2017). However, this approach may lead to surgical treatment of hyperparathyroidism that may resolve with long term follow-up (Yarlagadda

et al, 2017). If cinacalcet has been tried, and if this group of patients have persistent and significant hyperparathyroidism by 6 to 12 months, they should be referred for parathyroidectomy.

Chapter 7: Surgical techniques for parathyroidectomy

Since the 1960s, there has been ongoing discussion on the most optimal surgical procedure for renal hyperparathyroidism. From the viewpoint of the patient, the most important goal of all modalities of treating hyperparathyroidism, including surgical treatment is the improvement of quality of life, and reducing mortality (Konturek et al, 2016). Thus, the overall goal is resolution of symptoms of hyperparathyroidism, at the same time, ensuring normal calcium and phosphorous metabolism in patients with end stage renal disease. The objectives of surgical treatment should thus strive for an appropriate balance between the extent of parathyroidectomy, prevention of recurrent disease and avoidance of postoperative permanent hypoparathyroidism (Konturek et al, 2016). There are three surgical procedures for parathyroidectomy: (1) subtotal parathyroidectomy (ST-PTX), (2) total parathyroidectomy with auto transplantation (TPTX+AT) and (3) total parathyroidectomy without auto transplantation (TPTX). No definite evidence exists to date in favour of one over the other, as all of the above objectives cannot be guaranteed with any one of the techniques (Schneider et al, 2014). Often, the choice of procedure is determined solely by surgeon/institutional preference.

If an inferior parathyroid gland cannot be located, it is most likely located in one of the intra-thymic locations. Thymectomy is an important component of parathyroid surgery for end stage renal failure patients who have missing inferior parathyroid glands because retained intrathymic glands can be the source of persistent or recurrent disease (Milas, 2016). Concurrent thymectomy is also considered due to the presence of supernumerary glands and “rests” in the thymus. In one study, adding a cervical thymectomy increased the overall cure rate of hyperparathyroidism from 89 to 94 per cent (Boltz et al, 2015).

In regards to intraoperative PTH monitoring, an established protocol does not exist for renal hyperparathyroidism, due to delayed renal clearance of PTH (unlike the Miami Protocol for primary hyperparathyroidism). However, intraoperative monitoring is still used in some institutions to provide assurance that all hyper-functioning parathyroid tissue has been removed. In one study, a PTH decline of 80% predicted cure (Ohe et al, 2013), and a post resection PTH level that is high or fails to reduce suggests the presence of additional abnormal glands which warrants further resection.

7.1. Subtotal parathyroidectomy

ST-PTX is the resection of approximately 3.5 parathyroid glands, leaving behind 40-80mg (roughly the size of two normal parathyroid glands) of the most normal appearing parathyroid gland, well vascularized in situ after

marking it with a non-absorbable suture, or a metallic clip. Sometimes, a 75% resection of the remnant gland (near total parathyroidectomy) has been used, and a more aggressive resection could be attempted when parathyroid cryopreservation is available (Milas, 2016). Often, it should be combined with bilateral cervical thymectomy, to remove potential supernumerary parathyroid glands, which is more common in end stage renal patients in an environment of constant PTH stimulus. It appears preferable to save an inferior gland as they are usually located more anteriorly in the neck making the remnant more accessible to reoperation if necessary (Lorenz et al, 2015). The advantages of this technique include preservation of hormonal function by the parathyroid with normal blood supply in situ and low rate of postoperative permanent hypocalcaemia. Hence, some surgeons prefer ST-PTX over others in patients who have secondary hyperparathyroidism, who are expected to receive a renal transplant in the short term. In the case of patients who have a functioning renal transplant with tertiary hyperparathyroidism, it provides favourable conditions for such procedure, negating any possibility of damage to the transplanted kidney from sudden removal of PTH, as well as reducing the risks of iatrogenic hypoparathyroidism and hypocalcaemia (Konturek et al, 2016, Lorenz et al, 2015). The major disadvantages of ST-PTX is a risk of requiring reoperation in a technically difficult, scarred up neck when recurrent hyperparathyroidism occurs, as well as the necessity of general anaesthesia

and the increased risk of recurrent laryngeal nerve palsy/permanent damage from loss of tissue planes (Rothmund et al, 1991). A recent meta-analysis demonstrated that a need for reoperation in ST-PTX was 8.1%, which was not significantly different to TPTX + AT (6.6%) (Chen et al, 2016). Another potential risk is of parathyroid necrosis of the remnant, leading to hypoparathyroidism.

7.2. Total parathyroidectomy with auto-transplantation

Some surgeons perform TPTX + AT to reduce the chance of long term hypoparathyroidism and the need for long term calcium and vitamin D supplementation, as well as to avoid a difficult reoperation in the neck induced by hyper-function of autografted parathyroid tissue (Xu et al, 2016). This procedure aims to remove all parathyroid glands that can be identified and simultaneously auto transplants a small portion of a gland to a new, non-anatomic site (such as into a pocket created in the brachio-radialis or sternocleidomastoid muscles). Often, this procedure is accompanied by bilateral cervical thymectomy to ensure removal of supernumerary glands and parathyroid rests. The disadvantage is that there is the potential for profound hypoparathyroidism and hypocalcaemia if the autograft fails to revascularize (Milas, 2016). Auto-transplantation involves mincing a portion of the most normal appearing excised gland into 1-2mm pieces (a total of 10 to 15 pieces, or into slurry) which are then inserted into several muscular or subcutaneous pockets (Milas, 2016). Variations of this technique have been

described, including inserting each piece into an individual pocket, inserting several pieces into a pocket or injecting a suspension of fragments into a muscle with a syringe (Echenique-Elizondo et al, 2007, Milas, 2016). The brachio-radialis muscle of the non-dominant forearm, sternocleidomastoid muscle, pre-sternal chest as well as the subcutaneous fat of the abdomen are potential sites for re-implantation. The advantage of implantation in the non-dominant forearm is that re-exploration (if needed) can be readily carried out under local anaesthesia (Milas, 2016). It also provides the ability to determine if an autograft is functional by comparing PTH levels of blood samples drawn from the antecubital vein of the implanted arm versus the contralateral arm (Milas, 2016). Sternocleidomastoid muscle implantation has an advantage as it is in the operative field of parathyroid surgery. However, compared to forearm autografts, neck autografts are more difficult to retrieve at reoperations, and could potentially confound imaging studies performed in search of supernumerary glands in the neck (Milas, 2016). In all cases, the site of reimplantation should be marked with metallic clips or non-absorbable sutures so that it can be identified in case of re-intervention (Lorenz et al, 2015). The only prospective randomized trial comparing the outcomes of 40 patients with either TPTX + AT and ST-PTX demonstrated significantly reduced rates of recurrence, significantly more often normalization of serum calcium and alkaline phosphatase as well as

significant improvement of clinical signs like pruritus and muscle weakness in the TPTX + AT group (Rothmund et al, 1991). Regarding recurrence rates, several retrospective case and cohort series report 0-85% for TPTX + AT (Lorenz et al, 2015).

7.3 Total parathyroidectomy without auto-transplantation

During this procedure, at least four parathyroid glands are resected, and unilateral thymectomy is usually performed if fewer than two glands are detected at regular positions at the respective side. It aims for near complete abolishment of PTH, and is widely practiced in countries such as Japan (Lumachi and Basso, 2014). The concept offers the advantage of a highly standardized procedure with smaller uncertainty regarding adequate size and function of parathyroid remnants (Lorenz et al, 2015). Because TPTX has the highest risk of causing permanent hypocalcaemia, permanent hypoparathyroidism and possibly adynamic bone disease, it is normally reserved for patients who suffer from severe complications of hyperparathyroidism due to lifelong dialysis (e.g. renal osteodystrophy) and preferably with facilities for parathyroid cryopreservation if possible (Milas, 2016). TPTX should be generally contraindicated in patients who have a better chance of receiving renal transplantation due to iatrogenic hypoparathyroidism and hypocalcaemia post transplantation (Komaba et al, 2017). In a recent randomized trial of 100

patients comparing TPTX + AT and TPTX, at three years post-operative, patients who had TPTX had lower PTH levels (31.7 +/- 43.6 vs 98.2 +/- 156.8 pmol/l) and lower rates of persistent (1.9 vs 4.1%) or recurrent (0 vs 8.3%) hyperparathyroidism (Schlosser et al, 2016). A small scale retrospective study on 23 patients with TPTX demonstrated a better biochemical cure than ST-PTX (74% vs 63%) after median follow-up of 72 months (Lorenz et al, 2006). Contrary to the arguments regarding imminent development of adynamic bone disease and difficult medical management after TPTX, follow-up after two years demonstrated no clinical evidence of adynamic bone disease. The possible higher risk of developing adynamic bone disease and secondary calciphylaxis from calcium therapy must be carefully determined during long term follow-up in patients devoid of PTH excretion (Lorenz et al, 2015, Lorenz et al, 2006). However, presently, data is limited to evaluate merits and risks of this procedure to recommend it outside of controlled trials (Lorenz et al, 2015).

PART III: RETROSPECTIVE COHORT STUDY OF PATIENTS WITH HYPERPARATHYROIDSM IN END STAGE RENAL FAILURE

Chapter 8: Cinacalcet is associated with severe intraoperative and post-operative acute hyperkalaemia associated with hypocalcaemia after parathyroidectomy for renal hyperparathyroidism

Abstract

Objective: An impression that patients with prior treatment with cinacalcet and undergoing parathyroidectomy for renal hyperparathyroidism had greater acute hyperkalaemia and hypocalcaemia intraoperatively and immediate postoperative period prompted this study.

Methodology: A single institution study was performed between January 1993 and September 2014. Sixteen patients were on cinacalcet prior to parathyroidectomy, and 88 patients were controls.

Results: 81.25% of the cinacalcet group recorded an intraoperative or 24 hour postoperative potassium value ≥6.00mmol/L compared to 1.67% in the controls (P<0.001). 75% of cinacalcet patients had a potassium rise of ≥1mmol/L from preoperative value compared to 10% (P<0.001). This was correlated with greater median percentage rise of potassium from

preoperative value in the cinacalcet group (33.33% vs. 11.54%, P<0.001). Cinacalcet patients also had greater degree of hypocalcaemia 24 hours postoperatively (2.10 ± 0.23 vs. 2.25 ± 0.26 mmol/L, (P< 0.01)) and greater need for intravenous calcium (93.75% vs. 50%, (P < 0.001)).

Conclusions: This is the first report of acute rise in potassium seen in the intraoperative/immediate postoperative periods following parathyroidectomy in patients on cinacalcet for renal hyperparathyroidism. Clinicians should be alerted to this risk of hyperkalaemia so as to prevent morbidity and mortality.

Key words: cinacalcet, hyperkalaemia, hypocalcaemia, parathyroidectomy, renal hyperparathyroidism.

8.1 Introduction

Secondary and tertiary hyperparathyroidism are frequently observed in patients with end-stage renal failure (ESRF). The progressively falling glomerular filtration rate results in hyperphosphataemia, hypocalcaemia and reduced levels of circulating 1, 25-dihydroxyvitamin D3 (calcitriol) due to renal failure (Ghani and Baxter, 2012). The latter leads to increase secretion of parathyroid hormone (PTH) and diffuse parathyroid hyperplasia (Wirowski et al, 2013, Meyers et al, 2009). Eventual progression leads to the development of nodular hyperplasia, and a decrease in the number of vitamin D receptors and calcium sensing receptors (Meyers et al, 2009). This hyperparathyroidism results in increased morbidity and mortality from renal

osteodystrophy, soft tissue and vascular calcifications, dyslipidaemia, hypertension and cardiomyopathy (Ghani and Baxter, 2012). Currently, hyperparathyroidism from ESRF is predominantly managed medically, with a combination of phosphate binders, active vitamin D analogues, calcium and/or calcimimetics. Approximately 29% of ESRF patients undergo parathyroidectomy (PTX), which is primarily reserved for symptomatic patients with elevated PTH despite optimal medical therapy (Bajaj et al, 2011, Lafrance et al, 2013). Procedures such as subtotal and total PTX with or without sternocleidomastoid or forearm autograft continue to be the primary therapeutic options for refractory cases in Australia, Europe and United States (Zitt et al, 2011). The definitive treatment is renal transplantation; however, in nearly a third of these patients, the hyperparathyroidism is not resolved and persists even several years after transplantation, occasionally requiring PTX (Guerra et al, 2011). Cinacalcet hydrochloride (cinacalcet), also known by its trade name Sensipar (Amgen, Thousand Oaks, CA) is a calcimimetic that has been approved for use in secondary hyperparathyroidism since 2004 (Norman et al, 2012). It reduces PTH secretion by acting as a calcium receptor agonist as well as by increasing the sensitivity of calcium-sensing receptors, upregulating calcium-sensing receptor expression, and inhibiting parathyroid cell proliferation (Zitt et al, 2011, Tsuruta et al, 2013). Cinacalcet is reported to be generally well tolerated and labelled the new “medical parathyroidectomy” with nausea,

vomiting and diarrhoea as the most frequent adverse reactions (Ghani and Baxter, 2012, Wirowski et al, 2013). However, some patients who have been on cinacalcet discontinue their therapy due to adverse reactions or are resistant to its effects, potentially due to more aggressive disease and require PTX. The present study arose from observations that patients who were on cinacalcet for renal hyperparathyroidism and had subsequently undergone PTX developed more severe acute hyperkalaemia in the intraoperative and postoperative periods compared with those who were operated with traditional medical therapy. Specifically, the authors sought to determine whether there was any difference between these two groups of patients and if so, whether there was any correlation in the degree of intraoperative and postoperative hyperkalaemia and hypocalcaemia.

8.2 Patients and Methods

8.2.1. Study Population

A retrospective, single institution review of all patients who underwent PTX for hyperparathyroidism was conducted at The Canberra Hospital, Australian National University Medical School from January 1993 to September 2014. Only patients who underwent PTX for renal hyperparathyroidism were eligible for inclusion in the study. At this institution, PTX was only offered if patients failed optimal medical management (which included calcitriol, calcium carbonate and phosphate binders). The exclusion criteria in this

study were (1) primary hyperparathyroidism; (2) subtotal parathyroidectomy (to negate the effect of residual PTH secretion on calcium measurements); (3) previous parathyroid surgery; (4) histopathological diagnosis other than hyperplasia, such as parathyroid carcinoma, (5) patients who are not dialysis dependent and have functioning renal transplant and (6) patients where potassium was administered in the intraoperative/postoperative day 0 period.

Of the 386 patients who underwent PTX in the study period, 147 had renal hyperparathyroidism of whom 133 were operated by the first author. Of the 104 patients that met the inclusion criteria, 88 were controls on traditional medical therapy such phosphate binders, active vitamin D analogues and/or calcium, and 16 had cinacalcet on top of traditional therapy prior to PTX, and these patients were operated between 2007-2014. The charts were studied for the following variables: age, sex, type of procedure, histopathology, total weight and volume of the parathyroid glands (volume was calculated using the formula for an ellipsoid Vol=4/3 πabc. Laboratory parameters analysed in the perioperative period included 25-hydroxy vitamin D, PTH, potassium, corrected calcium, phosphate, magnesium, urea, creatinine and alkaline phosphatase until 144 hours post-operative. Generally, preoperative measurements were taken within 12-24 hours before surgery, intraoperative values were taken every half hourly when recorded, and postoperative values were taken 6-8 hourly till 48 hours postoperative and 8-24 hourly for postoperative 48 hours to 144 hours.

Patients were dialysed for medical optimization one day prior to PTX and for most cases, the preoperative biochemical variables were taken some hours after this. Patients who were on cinacalcet had their medication variably discontinued prior to surgery. Total PTX with auto-transplant to sternocleidomastoid/brachioradialis muscle was complete in the study population. Anaesthesia for PTX did not differ significantly between patients and followed anaesthetic protocol: induction drugs were generally propofol, fentanyl and atracurium; maintenance was achieved with oxygen/sevofluorane mixture, fentanyl was given intraoperatively for analgesia and glycopyrrolate/neostigmine at the end of surgery. Insulin/dextrose and/or calcium gluconate/chloride were given intraoperatively and resonium (a cation exchange resin for hyperkalaemia) was given postoperatively when potassium was greater than 6.00mmol/L. Postoperatively, patients were on varying doses of calcitriol and/or calcium carbonate three to six hourly, depending on the severity of hypocalcaemia and at the discretion of the surgeon. In addition, if corrected calcium was less than 1.9mmol/L, intravenous (IV) calcium gluconate/chloride was infused for one to two hours and repeated whenever indicated, and was used as a proxy for profound hypocalcaemia.

8.2.2. Statistical analysis

According to the variable distribution, a Student t test was used to compare the groups. A chi-square test was used to compare proportions (percentages).

Continuous variable data were reported as mean ± standard deviations (SD). For all analysis, p value ≤0.05 was considered statistically significant. All analyses were done using SPSS Statistics Software, and Microsoft Excel 2010 (Redmond, WA, USA).

8.3 Results

8.3.1. Baseline characteristics

The baseline characteristics, including patient demographics and preoperative laboratory data of control and cinacalcet groups are presented in **Table 1**. There was no significant difference in gender, however the cinacalcet group were significantly younger (44.38 ± 14.22 years) than the controls (52.30 ± 14.24 years). All other major preoperative variables were not significant between the two groups.

8.3.2. Cinacalcet is associated with a greater rise in potassium in the intraoperative and the first 24 hour postoperative periods

Figure 20 demonstrates a scatter plot of preoperative and the highest intraoperative/24 hour postoperative potassium concentrations in the cinacalcet and control patients. Twenty eight out of eighty eight patients from the control group did not have intraoperative or postoperative day 0 potassium values recorded. 81.25% (n=13/16) of the cinacalcet group recorded an intraoperative or 24 hour postoperative potassium value

≥6.00mmol/L, compared to 1.67% (n=1/60) in the controls (P<0.001). More importantly, 43.75% (n=7/16) of the cinacalcet group registered an intraoperative or 24 hour postoperative potassium value ≥7.00mmol/L whereas no control patients reached this value. Overall, 75% (n=12/16) of cinacalcet patients had a potassium rise of ≥1mmol/L from preoperative value compared to 10% (n=6/60) (P<0.001). The average change in potassium from preoperative and intraoperative/24 hour postoperative in the cinacalcet group was +1.49 ± 0.92mmol/L versus +0.15 ± 0.63mmol/L, (P<0.001).

The percentage change of serum potassium from preoperative to highest intraoperative/24 hour postoperative of those that had a positive change in the cinacalcet (n=15/16) and control patients (n=37/60) is illustrated as a Box and Whisker Plot in **Figure21**. Overall, the positive percentage change was greater in the cinacalcet group compared to controls (P<0.001). The values ranged between 5.88-69.05% in the cinacalcet and 0-38.89% in the controls. The 25^{th}, 50^{th} and 75^{th} percentile changes of the cinacalcet patients was 18.59%, 33.33% and 43.92% respectively compared to 5.88%, 11.54% and 15.38% respectively for the control patients.

Table 2 provides detailed information of patient characteristics, serial potassium levels and interventions undertaken for each of the cinacalcet patients. Typically, these patients have been on cinacalcet for 19.18 ± 16.66 months at a dose between 30-180mg/day, at the discretion of the

nephrologist. Eight patients had intraoperative potassium readings. Notably, patient 6 registered potassium of 7.10mmol/L after removal of the fourth parathyroid gland and was given 10 units of insulin, 50% dextrose in 50ml and 10ml of 10% calcium gluconate. Patient 12 had the first intraoperative potassium reading of 6.40mmol/L at the time of removal of first parathyroid gland at which 50% dextrose and 10 units of insulin in 50ml were given 30 minutes later. Despite this, the potassium level elevated to 6.70mmol/L by the time of removal of the fourth parathyroid glands requiring further IV 50% dextrose and 10 units of insulin in 50ml as well as 10mls of calcium chloride (6.80mmol/L) was given. Patient 15 had an intraoperative potassium of 6.80mmol/L after the removal of the fourth parathyroid gland, and was given 10 units of insulin and 50% dextrose in 50ml. Two patients recorded peaked electrocardiogram *T*-waves: patient 6, during the intraoperative period and patient 15 seven hours after PTX. All of the cinacalcet patients recorded acute hyperkalaemia in the postoperative period, during which 15/16 patients required dialysis in the first 24-48 hours postoperative period, and all patients were given a combination of dextrose, insulin and/or IV calcium and in some patients, resonium.

Figure 22 demonstrates the relationships between preoperative PTH (provided as four quartiles) and mean highest intraoperative/24 hour postoperative potassium with respect to the proximity cinacalcet was ceased prior to surgery. There appeared to be no obvious relationship between (1)

PTH quartiles and the serum potassium levels in the group representing proximal cessation of cinacalcet and (2) serum potassium levels in the highest PTH quartile between the groups representing proximal and distal cessation of cinacalcet. This suggested that intraoperative PTH and the proximity of cessation of cinacalcet prior to surgery were probably independent variables.

8.3.3. Cinacalcet is associated with greater need for IV calcium postoperatively and prolonged hospital stay

Significantly greater proportion of cinacalcet patients required IV calcium to control hypocalcaemia in the postoperative period than control patients (93.75% vs. 50%, ($P < 0.001$)) **(Table 1)**. The postoperative hospital stay was extended in the cinacalcet patients (7.73 ± 2.73 vs. 5.81 ± 2.76 days, ($P <0.01$)) due to complex management of prolonged hypocalcaemia, despite medical intervention.

8.3.4. Cinacalcet is associated with greater hypocalcaemia and increased bone turnover

Cinacalcet patients had lower mean preoperative corrected calcium level (2.49 ± 0.32 vs. 2.57 ± 0.20 mmol/L, ($P>0.05$)) and had larger percent reduction 24 hours after operation compared to controls (15.56% vs 11.95%) **(Figure 23)**. The cinacalcet group maintained hypocalcaemic levels (normal lower range 2.10 mmol/L) 24 hours (2.10 ± 0.23 vs. 2.26 ± 0.26 mmol/L,

(P< 0.01)), 48 hours (1.90 ± 0.20 vs. 2.13 ± 0.26 mmol/L, (P<0.001)), 72 hours (2.09 ± 0.21 vs. 2.20 ± 0.29 mmol/L, (P>0.05)), 96 hours (2.02 ± 0.20 vs. 2.21 ± 0.29 mmol/L, (P<0.01)) and 120 hours (2.10 ± 0.17 vs. 2.23 ± 0.31 mmol/L, (P>0.05)) after surgery relative to controls, most of whom maintained normocalcaemia with oral calcium and calcitriol in the postoperative period.

Alkaline phosphatase concentrations between preoperative and postoperative day 5 demonstrated an overall increasing trend in both groups following PTX. Alkaline phosphatase levels in the cinacalcet group was greater during the preoperative (324.25 ± 264.98 vs. 239.51 ± 219.10 U/L, P>0.05)) and postoperative periods at 24 hours (329.79 ± 301.69 vs. 198.27 ± 136.36 U/L, (P<0.05)), 48 hours (317.31 ± 292.82 vs. 231.24 ± 205.98 U/L, (P>0.05)), 72 hours (329.42 ± 302.70 vs. 229.09 ± 188.06 U/L, P>0.05)), 96 hours (396.8080 ± 390.81 vs. 300.77 ± 278.63 U/L, P>0.05)), and 144 hours (502.23 ± 491.71 vs. 375.41 ± 377.75 U/L, P>0.05)).

8.4 Discussion

Cinacalcet, a class II calcimimetic drug that reduces production of PTH, gained approval from the Food and Drug Administration for use in patients with ESRF in March 2004, and is well studied in this group of patients (Lazar and Stanus, 2007, Urena et al, 2009, Bover et al, 2009). In Australia, following approval for its use soon after that, the number of patients with

ESRF referred for surgical PTX fell. This was best demonstrated by the number of patients operated in our institution in the past eight years, whereby eighteen patients were operated for renal hyperparathyroidism in 2005 versus only five patients in 2013. This has also been observed in other institutions (Lafrance et al, 2013, Cunningham et al, 2005, Li et al, 2011). In this institution, patients waiting for PTX nowadays had been exposed to cinacalcet for varying periods since 2007.

After PTX, the study group demonstrated more severe and prolonged hypocalcaemia and acute hyperkalaemia, greater requirement for insulin/dextrose and IV calcium. Sudden removal of PTH by PTX appeared to severely diminish mobilisation of skeletal calcium with resultant hypocalcaemia, and an associated acute rapid rise of serum potassium, the cause of which is undetermined. It was expected the level of serum potassium would have risen higher without the use of insulin/dextrose.

Critical incident review, in one patient, of the events during surgery and the immediate post- operative day, clearly demonstrated that some patients treated previously with cinacalcet may respond differently from those not exposed to the medication **(Table 2)**. This patient developed severe intraoperative hyperkalaemia of 7.10mmol/L with tall peaked *t*-waves. This required urgent IV insulin/dextrose in the operating room twice to reduce the serum potassium. This level of acute hyperkalaemia occurred as soon as the fourth parathyroid gland was removed. The arterial blood gas demonstrated a

severe fall of ionised calcium and three doses of calcium chloride were given IV to correct this deficit. Four hours after the surgery, the patient redeveloped hyperkalaemia, requiring immediate haemodialysis.

Suspecting that this pattern of electrolyte changes occurred more commonly with patients treated with cinacalcet previously, the authors reviewed the literature and found a report of mortality in a patient undergoing PTX for secondary hyperparathyroidism due to ESRF (Zahoor et al, 2012). This patient was one of a cohort of patients reported in a subsequent series demonstrating marginally more severe hyperkalaemia following PTX. Unfortunately, no data was available in that paper on the status of whether patients had been treated with cinacalcet prior to PTX, nor the serum calcium concentrations intraoperatively and postoperatively (Bajaj et al, 2011, Zahoor et al, 2012).

Meyers et al noted similar findings whereby in patients who had undergone PTX, cinacalcet use preoperatively led to lower postoperative calcium and greater requirements for IV calcium infusion postoperatively compared with those who did not receive it (Meyers et al, 2009). However, they did not comment on the more significant finding of severe hyperkalaemia as had been reported in one other study (Bajaj et al, 2011, Zahoor et al, 2012).

The results from our cohort of sixteen patients showed that patients treated with cinacalcet had greater reductions in serum calcium after PTX, and

required more IV calcium, with their hospital stay significantly longer. The most significant change however was a potassium elevation of > 6.00mmol/L observed in 81.25% (n=13/16) of these patients, even as soon as after removal of the first two parathyroid glands, and more so with all parathyroid glands removed. This hyperkalaemia may lead to cardiac arrhythmia, possibly accounting for the death reported in one study (Bajaj et al, 2011, Zahoor et al, 2012). Our study shows how it is critical to closely monitor patients undergoing PTX, irrespective of whether they had been treated with cinacalcet or not, as in the earlier years, some patients not on cinacalcet reported hyperkalaemia. However, the incidence was much lower as seen in our data and other studies (Bajaj et al, 2011, Cruz and Perazella, 1997). Between the two groups (other than the introduction of cinacalcet) there does not appear to be any major changes to the management of renal hyperparathyroidism. There were no changes in dialysis schedules (as the patients were appropriately dialysed 1 day prior to operation), the anaesthetic drugs used peri-operatively were on the whole, consistent, and the management of hyperkalaemia followed local protocol, which had not altered in the study period.

The results of this study suggest that the two events, hyperkalaemia and hypocalcaemia, may be interrelated. Although there is no confirmation, the authors propose that the cinacalcet patients had more profound hungry bone syndrome following parathyroidectomy. This is probably, in part, due to this

group of patients having greater duration of dialysis prior to surgery **(Table 1)**, perhaps due to the partial control of PTH levels, delaying referral for parathyroidectomy by the nephrologists. During dialysis, it is proposed that the bones probably underwent further demineralization. Thus, at the time of surgery, rapid onset of hypocalcaemia triggered by parathyroidectomy from severe hungry bone syndrome may have led to efflux of potassium from the cells, to maintain electrical neutrality. We put forward the view that cinacalcet should be ceased before surgery, although the number of weeks prior to PTX when cinacalcet should be ceased is uncertain.

Cinacalcet has a half-life of 30-40 hours, is metabolized by the cytochrome P450 mixed oxidase enzyme system and 80% of the metabolites are excreted by the kidneys (Poon, 2005). The metabolites are inactive and not thought to affect calcium receptors. The study results show the association with cinacalcet exposure prior to surgery, but cast little insight as to mechanism of hyperkalaemia and hypocalcaemia. .This is shown in Table 3 whereby the highest intraoperative/24 hours postoperative potassium were not related to either the preoperative PTH value, nor proximity of cessation of cinacalcet. Nonetheless, hyperkalaemia occurred as early as the removal of the first hyperplastic parathyroid gland and continued as more glands are removed.

Surgical practice has altered at The Canberra Hospital where there is now a policy of intraoperative monitoring in all patients undergoing PTX for ESRF. We believe this has prevented unnecessary morbidity and possibly mortality by identifying sudden elevations of serum potassium in the intraoperative and immediate postoperative periods (**Table 3**).

Although our findings are consistent in 16 cases, and are of clinical practice importance, we acknowledge the limitations of this study.

The sample size of the cinacalcet group was small in comparison to the control group. Postoperative serum potassium and calcium measurements were analysed at different times in the postoperative period. Episodes of hyperkalaemia or hypocalcaemia may have been overlooked. There was no intraoperative or immediate postoperative potassium recorded in some of the control patients, as hyperkalaemia in this group was not suspected. Lastly, the study was limited up to 144 hours postoperative period.

8.5 Conclusions

This is the first observational report of acute rise in potassium associated with hypocalcaemia observed in the intraoperative and immediate

postoperative period in cinacalcet patients who have had PTX for renal hyperparathyroidism.

The mechanism is unknown. Future basic sciences, or clinical investigations to demonstrate a direct relationship between cinacalcet, hypocalcaemia, and hyperkalaemia would be required to explain this observation. Calcimimetic use is likely to increase world-wide, in patients with ESRF. Some of these patients on cinacalcet, or who have been treated with cinacalcet, will require PTX. Awareness of this phenomenon of severe hyperkalaemia associated with hypocalcaemia by surgeons, anaesthetists and nephrologists will minimise patient risk.

8.6 Figures and Tables

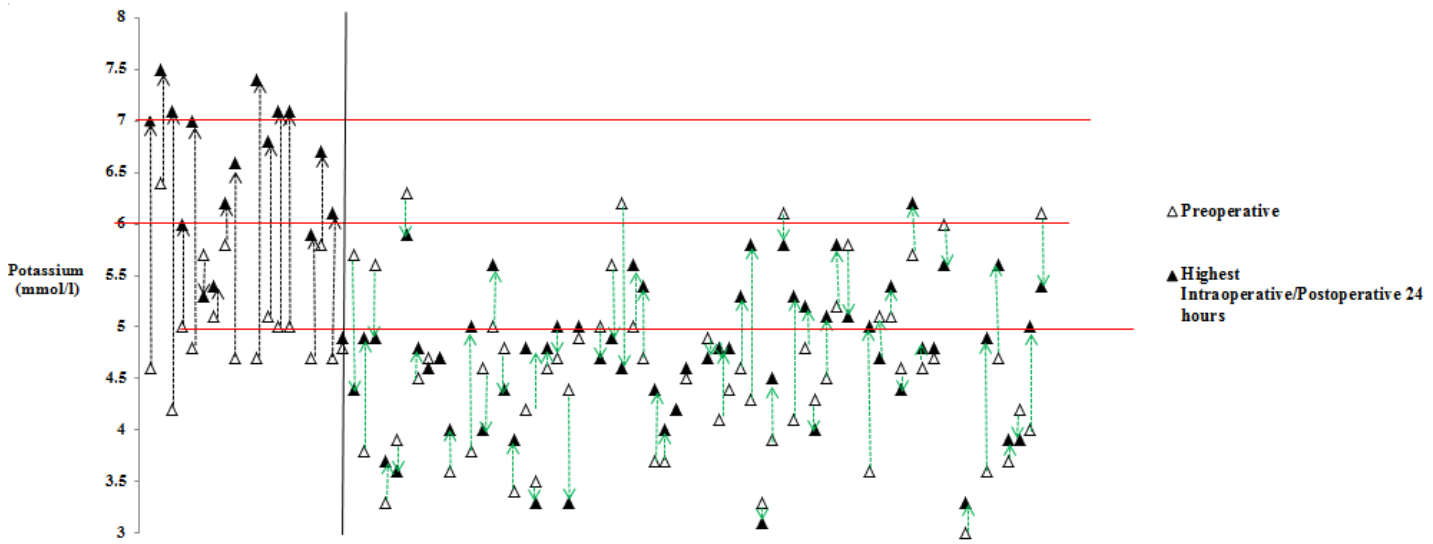


Figure 20. Preoperative and highest intraoperative/postoperative day 0 potassium values of cinacalcet and control patients. Horizontal axis denotes patients. Vertical dotted line delineates cinacalcet patients to the left, and control patients to the right. Horizontal dotted lines mark potassium concentration of 5.00mmol/L, 6.00mmol/L and 7.00mmol/L. Arrows indicate potassium concentration change between preoperative and intraoperative/postoperative day 0 values.

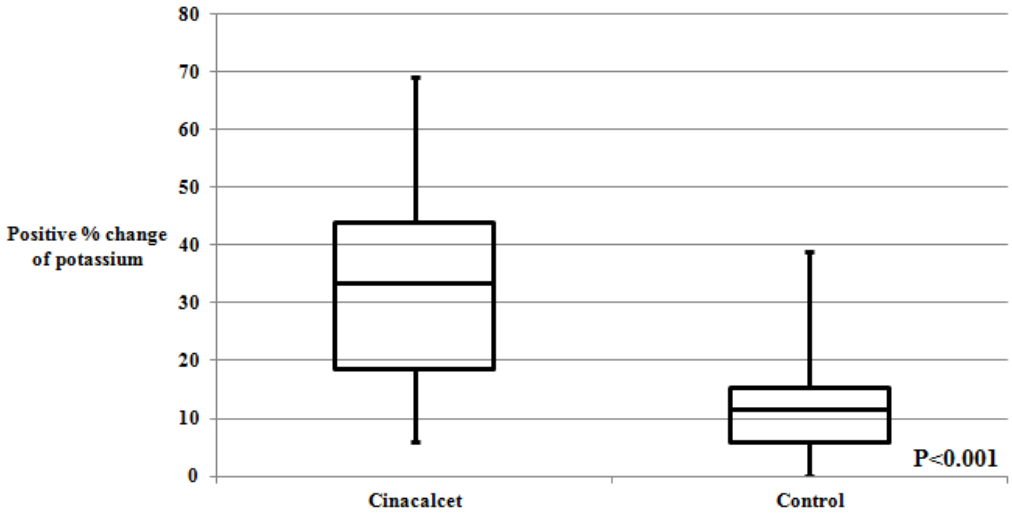


Figure 21. Box and Whisker Plots of positive percentage change in serum potassium from preoperative to highest intraoperative/postoperative day 0 in cinacalcet and control patients.

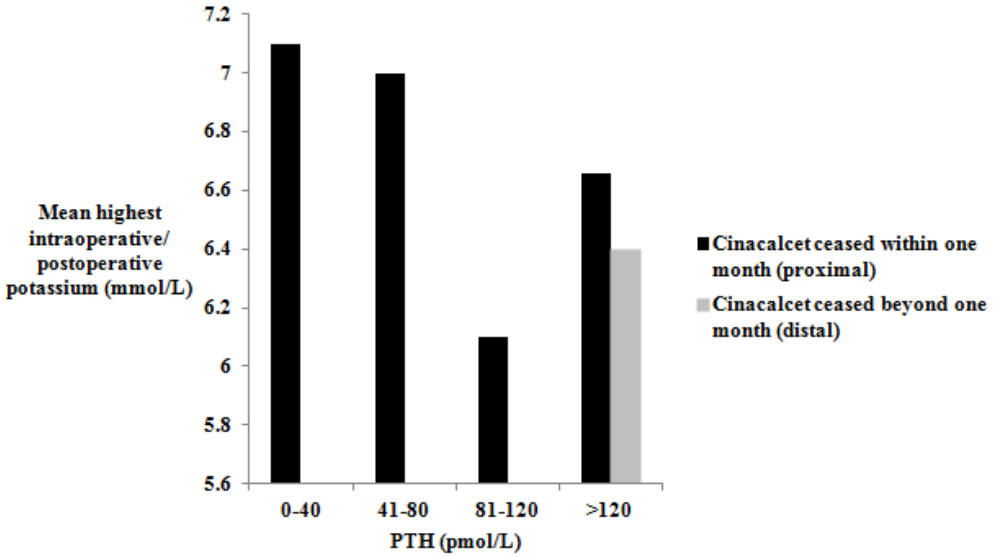


Figure 22. Mean highest intraoperative/postoperative 24 hour potassium between preoperative PTH quartiles in relation to proximity of cessation of cinacalcet prior to PTX

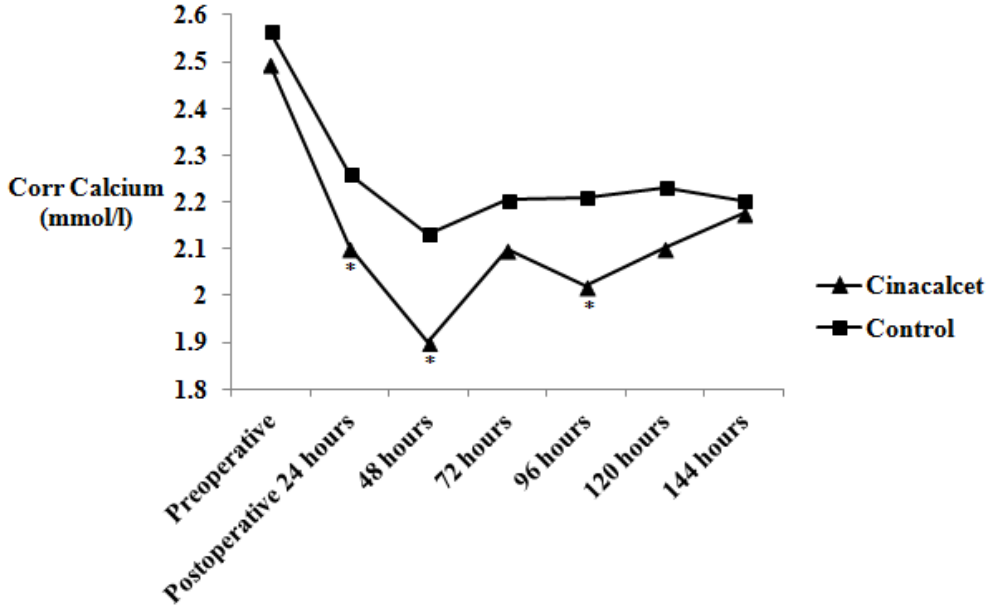


Figure 23. Preoperative and lowest postoperative corrected calcium values in control and cinacalcet patients. *Vertical bars* represent standard error. * P<0.05 versus controls.

Table 1. Patient demographics, preoperative laboratory and perioperative characteristics.

Characteristic	Total (n=104)	Cinacalcet (n=16)	Control (n=88)	P Value
Age (years)	51.08 ± 14.45	44.38 ± 14.22	52.30 ± 14.24	<0.05
Sex (M:F)	54:50	10:6	44:44	NS
Duration of dialysis (years)	4.60 ± 2.79	4.50 ± 2.83	3.98 ± 2.79	NS
25-hydroxy vitamin D (ng/ml)	62.00 ± 19.36	58.67 ± 21.39	72.00 ± 8.49	NS
Calcium corrected (mmol/L)	2.55 ± 0.22	2.49 ± 0.32	2.57 ± 0.2	NS
Phosphate (mmol/L)	1.99 ± 0.58	2.02 ± 0.53	1.99 ± 0.59	NS
Magnesium (mmol/L)	0.89 ± 0.19	0.97 ± 0.11	0.87 ± 0.20	NS
Urea (mmol/L)	19.49± 8.15	20.76 ± 10.98	19.26± 7.58	NS
Creatinine (umol/L)	785.52 ± 307.75	859.00 ± 349.07	772.01 ± 299.81	NS
Alkaline Phosphatase (U/L)	252.80 ± 227.57	324.25 ± 264.98	239.51 ± 219.10	NS
Volume of hyperplasia (mm^3)	22210.97 ± 18062.14	14628.77 ± 9876.17	23533.44 ± 18863.53	<0.05
Total weight of glands (g)	2.55 ± 1.78	2.37 ± 1.70	2.60 ± 1.82	NS
IV calcium postoperatively	56.73%	93.75% (15/16)	50% (44/88)	<0.001
Postoperative stay (days)	6.10 ± 2.83	7.73 ± 2.73	5.81 ± 2.76	<0.01

Data presented as mean ± SD. Abbreviations: ESRF, End stage renal failure; IV, intravenous; NS, Not significant.

Patient	Renal transplant (RTX)	Cinacalcet duration (months)	Daily Cinacalcet dose (mg)	Cinacalcet taken off before surgery (months)	Pre - operative potassium (mmol/L)	Intra- operative potassium (mmol/L)	Post- operative potassium Day 0 (mmol/L)	First postoperative dialysis given	IV insulin/dextrose and/or IV calcium given
1	Nil	1.5	60	0	4.1	-	7.0	Yes- Within 24 hours	IV insulin/dextrose + IV calcium
2	Nil	22	90	0	5.0	7.1, 6.5, 5.7, 5.8	5.4, 6.0, 5.6, 6.1, 6.4, 4.9	Yes- Within 24 hours	IV insulin/dextrose + IV calcium
3	Nil	20	30	0	4.2	-	7.1, 6.4, 6.5, 4.4	Yes- Within 24 hours	IV insulin/dextrose + IV calcium
4	Nil	15	30	0	5.0	-	6.0	Yes- Within 48 hours	IV calcium only

5	Nil	5	-	0	5.7	-	5.3	Yes-	Within 48 hours	IV calcium only
6	Nil	10	150	0.25	4.7	7.1	7.3, 7.4, 7.4, 7, 5.6, 5.0	Yes-	Within 24 hours	IV insulin/dextrose + IV calcium
7	Nil	12.5	180	0.5	5.8	-	6.2	Yes-	Within 48 hours	IV calcium only
8	Failed RTX	14	120	0.5	4.7	4.6, 5.1, 5	4.8, 5, 5.8, 6.1	Yes-	Within 48 hours	IV calcium only
9	Nil	68	180	1	5.0	6.0, 5.2, 5.0, 5.7, 5.6	5.5, 5.6, 7.1, 5.6, 6.2, 6.7	Yes-	Within 48 hours	IV insulin/dextrose + IV calcium
10	Nil	40	180	1	5.8	6.5, 6.2, 5.7, 5.6	6.7, 6.2, 5.0	Yes-	Within 24 hours	IV insulin/dextrose + IV calcium
11	Failed RTX	6	30	1	6.4	-	7.5, 6.8, 6.3,	Yes-	Within 24	IV insulin/dextrose + IV

							6.7, 3.9	hours	calcium
12	Failed RTX	14	30	1	5.1	6.4, 6.7, 5.6	5.6, 4.8, 6.8, 3.0	Yes- Within 24 hours	IV insulin/dextrose + IV calcium
13	Failed RTX	15	120	1	4.7	5.0, 5.0	5.1, 5.4, 5.3, 5.9, 4.7	Yes- Within 48 hours	IV insulin/dextrose + IV calcium
14	Nil	38	60	2	4.7	6.8	6.6, 6.4	Yes- Within 48 hours	IV insulin/dextrose + IV calcium
15	Nil	12	90	6	4.8	-	6.8, 7.0	Yes- Within 170 hours	IV insulin/dextrose + IV calcium
16	Nil	14	90	15	5.1	-	5.4	Yes- Within 48 hours	IV calcium only

Table 2. Perioperative characteristics of cinacalcet patients. Hyperkalaemia in our study is defined as >5mmol/L.

Table 3. Protocol for PTX in ESRF patients with renal hyperparathyroidism

1. Dialyse patients 24 hours before operation **2.** Check serum potassium, calcium, magnesium, phosphate and alkaline phosphatase prior to operation **3.** On the day of operation, place a central line or peripherally inserted central catheter, and an arterial line before surgery **4.** Continuously monitor ECG for tall peaked *t*-wave, shortened *QT* interval, lengthening of *PR* interval and *QRS* complexes intraoperatively **5.** Analyse arterial blood for potassium and ionised calcium hourly and to time with the removal of first and fourth parathyroid glands **6.** (a) Administer 10 units of IV insulin in 50% dextrose in 50ml if serum potassium >6.0mmol/L (b) Administer additional dose of IV 10% calcium gluconate in 10ml given over 10 minutes if (a) fails to reduce serum potassium level	**7.** Consider admitting patient to high dependency unit after operation **8.** Monitor calcium, magnesium, phosphate and potassium every two hours in the first 24 hours postoperative period, and four hourly between postoperative 24-48 hours period **9.** Administer oral calcium carbonate and calcitriol every three to six hourly and give bolus dose of IV calcium if corrected calcium < 1.90 mmol/L **10.** Consider dialysis in the first 24 hours if serum potassium is not well controlled by 10 units of IV insulin in 50% dextrose in 50ml and IV calcium.

.PART IV: THESIS SUMMARY AND CONCLUSIONS

Chapter 9: Summary and Conclusions

Parathyroid surgery has come a long way since its discovery just over 160 years ago. Sir Richard Owen is honoured for his discovery of parathyroid glands following several years of dissection in Rhinoceros carcasses between 1850-1852. Notable physicians and surgeons have subsequently contributed to the body of knowledge through trial and error, and driven by curiosity and determination. Regrettably, early human research and medical practice sometimes was associated with patient morbidity and mortality. The case of Captain Charles Martell is an exemplar of this, highlighting the natural course of parathyroid disease and associated manifestations (skeletal manifestations, nephrolithiasis), the need for better localisation tools of parathyroid glands and requirement of an experienced parathyroid surgeon (he underwent seven operations), the presence of ectopically located parathyroid glands (mediastinum), the associated lethal postoperative complications of parathyroidectomy (tetany and laryngospasm) and most importantly, the importance of involving patients in decision making (he had read about ectopically positioned parathyroid glands in the mediastinum in *Acta Medica Scandinavica* and managed to convince Cope and Churchill to explore his mediastinum just prior to his seventh and final parathyroid operation). Few patients in the annals of history have been studied as extensively as Captain Martell, who eventually succumbed from the natural course of his disease (nephrolithiasis).

There is a complex and systemic interplay between the parathyroid glands, bone, kidneys, intestines and skin on calcium, magnesium and phosphate homeostasis. Progress in basic biological science remains fundamental to foster clinical research and evidence based practice for better investigative tools, and treatment (medical and surgical. An example is the development of an immunoassay for measurement of parathyroid hormone by Berson and Yalow in 1963, which earned them the Nobel Prize in Physiology or Medicine. Another is the FDA approval in 2004 of cinacalcet as an alternative to parathyroidectomy in hyperparathyroidism.

The incidence and prevalence of dialysis dependant end stage renal failure is increasing in the Western societies, and consumes a significant quantum of healthcare resources. Although the optimal treatment of end stage renal disease is renal transplantation, there are issues with kidney donor shortage, and toxicity of immunosuppressive medications. Parathyroidectomy is a proven useful treatment prior to renal transplantation to alleviate the symptoms of renal osteodystrophy.

Following the introduction of cinacalcet, there has been a fall in the number of parathyroidectomy operations performed in our institution, and around the world. However, in patients exposed to cinacalcet and subsequently undergoing parathyroidectomy (due to refractory disease or cessation of cinacalcet due to side effects), we have demonstrated severe hyperkalaemia

and greater hypocalcaemia in the intraoperative and in the immediate postoperative period. The hyperkalaemia was significantly elevated to the extent of ECG changes, there was greater requirement of intravenous insulin/dextrose and for some, emergency dialysis. Cinacalcet patients required significantly greater amounts of intravenous calcium to correct hypocalcaemia. It remains speculative that the two events may be related perhaps to maintain serum electrical neutrality. Our findings resulted in a change in protocol for perioperative management of parathyroidectomy in renal hyperparathyroidism in our institution. Greater awareness from surgeons, anaesthetists and nephrologists is necessary to prevent morbidity and mortality in this group of patients.

PART V: REFERENCES

.

Abdelhadi M Nordenstrom. Bone mineral recovery after parathyroidectomy in patients with primary and renal hyperparathyroidism. J Clin Endocrinol Metab, 1998; 83: 3845.

Akerstrom G, Malmaeus J, Bergstrom R. Surgical anatomy of human parathyroid glands. Surgery, 1984; 95: 14-21.

Alkhalili E, Tasci Y, Aksoy E et al. The utility of neck ultrasound and sestamibi scans in patients with secondary and tertiary hyperparathyroidism. World J Surg, 2015; 39: 701-705.

Almaden Y, Hernandez A, Torregrosa V et al. High phosphate level directly stimulates parathyroid hormone secretion and synthesis by human parathyroid tissue in vitro. J Am Soc Nephrol, 1999; 9: 1845-1852.

Aly ZA, Gonzalez EA and Martin KJ. Parathyroid Hormone, Vitamin D and metabolic bone disease in dialysis patients. In Nissenson AR and Fine RN "Clinical Dialysis" Fourth Edition. The McGraw-Hill Companies, Inc, USA, 1995.

Anari H, Bashardoust B, Pourissa M et al. The Diagnostic accuracy of high resolution ultrasound imaging for detection of secondary hyperparathyroidism

in patients with chronic renal failure. Acta Medica Iranica, 2011; 49(8): 527-530.

Bajaj Y, Roberts S, Simon D, Snowden C, Gianopoulos I, England RJ. Intra-operative hyperkalaemia: a serious but under recognised complication of renal parathyroidectomy- a prospective study: how we do it. Clinical Otolaryngology, 2011; 36: 69-85.

Bann DV, Zacharia T, Goldenberg D, Goyal N. Parathyroid localization using 4D-computed tomography. Ear Nose Throat J 2015; 94(4-5): E55-57.

Barrett KE, Barman SM, Boitano S, Brooks HL.Ganong's Review of Medical Physiology Twenty Fourth Edition. McGraw-Hill Companies Inc, USA, 2012. Page 381-389.

Bentrem DJ, Angelos P, Talamonti MS et al. Is preoperative investigation of the thyroid justified in patients undergoing parathyroidectomy for hyperparathyroidism? Thyroid, 2002; 12: 1109.

Berkoben M, Quarles LD. Indications for parathyroidectomy in end stage renal disease. Up to Date, Wolters Kluwer, USA, 2015.

Berkoben M, Quarles LD. Pathogenesis of refractory hyperparathyroidism and indications for parathyroidectomy. In UpToDate, Wolters, Kluwer, Netherlands, 2016.

Bland-Sutton J. Tumours: Innocent and Malignant, Their Clinical Characteristics and Appropriate Treatment. 1st through 7th edition (1922). London: Cassell, 1893.

Bloom and Fawcett A textbook of histology-12th Edition. Chapter 19, Page 498-502.Taylor and Francis, United States of America, 1997.

Boltz MM, Zhang N, Zhao C et al. Value of prophylactic cervical thymectomy in parathyroid hyperplasia. Ann Surg Oncol, 2015; 22 Suppl 3: S662.

Borchhardt K, Sulzbacher I, Benesch T et al. Low turnover bone disease in running a is almost what they say is on his dancing is there not yours hypercalcemic hyperparathyroidism after kidney transplantation. Am J Transplant, 2007; 7: 2515.

Boron WF, Boulpael EL. Medical Physiology- Updated Edition. Elsevier-Health Sciences Division. Philadelphia, USA, 2012. Page 1090-1102.

Bover J, Aguilar A, Bass J et al. Calcimimetics in the chronic kidney disease-mineral and bone disorder. Int J Artif Organs, 2009; 32: 108-121.

Brown SJ, Lee JC, Christie J, Maher R, Sidhu SB, Sywak MS, Delbridge LW. Four-dimensional computed tomography for parathyroid localization: a new imaging modality. ANZ Journal of Surgery 2014; 85(6): 483-487.

Carney A. The glandular parathyroideae of Ivar Sandstrom. Am J Surg Pathol, 1996; 20: 1123-1144.

Carney JA. The glandulae parathyroideae of Ivar Sandstrőm: contributions from two continents. Am J Surg Pathol 1996; 20: 1123-1144.

Carter WB, Carter DL, Cohn H. Cause and current management of reoperative hyperparathyroidism. Am Surg, 1993; 59: 120.

Chen J, Jia X, Kong X et al. Total parathyroidectomy with auto transplantation versus subtotal parathyroidectomy for renal hyperparathyroidism: a systematic review and meta-analysis. Nephrology, 2016; doi: 10.1111/nep.12801. [Epub ahead of print].

Chou FF, Chen JB, Lee CH et al. Parathyroidectomy can improve bone mineral density in patients with symptomatic secondary hyperparathyroidism. Arch Surg, 2001; 136: 1064.

Coakley AJ, Kettle AG, Wells CP et al. 99mTc sestamibi a new agent for parathyroid imaging. Nucl Med Commun, 1989; 10: 791-794.

Coburn JW, Hartenbowe DL and Brickman AS. Advances in vitamin D metabolism as they pertain to chronic renal disease. American Journal of Clinical Nutrition, 1976; 29: 1283-1299.

Cruz DN and Perazella MA. Biochemical aberrations in a dialysis patient following parathyroidectomy. Am J Kid Dis, 1997; 29(5): 759-768.

Cruzado JM, Moreno P, Torregrosa JV et al. A Randomized study comparing parathyroidectomy with cinacalcet for treating hypercalcaemia in kidney allograft recipients with hyperparathyroidism. J Am Soc Nephrol, 2016; 27: 2487.

Cunningham J, Danese M, Olson K, Klassen P, Chertow GM. Effects of the calcimimetic cinacalcet HCl on cardiovascular disease, fracture and health-

related quality of life in secondary hyperparathyroidism. Kidney Int, 2005; 68(4): 1793-1800.

Cunningham J, Locatelli F, Rodriguez M. Secondary hyperparathyroidism: pathogenesis, disease progression, and therapeutic options. Clin J Am Soc Nephrol, 2001; 6: 913.

Davita- The history of dialysis, life, death and a "washing machine", 2017. Website:https://www.davita.com/kidneydisease/dialysis/motivational/the-history-of-dialysis/e/197. Last accessed: 26 October, 2017.

Dawson-Hughes B, Harris SS, Palermo NJ. Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women. J Clin Endocrinol Metab 2009; 94: 96-102.

De Andrade J, Mangussi-Gomes J, da Rocha L et al. Localization of ectopic and supernumerary parathyroid lands in patients with secondary and tertiary hyperparathyroidism: surgical description and correlation with preoperative ultrasonography and Tc99m-Sestamibi scintigraphy. Braz J Otorhinolaryngol, 2014; 80(1): 29-34.

Delbridge LW, Palazzo FF. First parathyroid surgeon: Sir John Bland-Sutton and the parathyroids. ANZ J. Surg 2007; 1058-1061.

Dhawan P, Peng X, Sutton AL et al. Functional cooperation between CCAAT/enhancer binding proteins and the vitamin D receptor in regulation of 25-hydroxyvitaminD3 24-hydroxylase. Mol Cell Biol, 2005; 25: 472-487.

Divieti P, Inomata N, Chapin K et al. Receptors for the carboxyl-terminal region of PTH (7-84) are highly expressed in osteocytic cells. Endocrinology, 2001; 142: 916-925.

Dolev E. A gland in a Search of a Function: The Parathyroid Glands and the Explanations of Tetany 1903-1926. Journal of History of Medicine and Allied Health Sciences, 1987; 42(2): 186-198.

DuBose, J, Morvant J. “Bodies So Tiny”: The History of parathyroid Surgery. Current Surgery 2005; 61 (1): 91-95.

Dulfer RR, Franssen GJ, Hesselink DA et al. Systematic review of surgical and medical treatment for tertiary hyperparathyroidism. BJS, 2017; 104: 804-813.

Dusso AS and Brown AJ. Chapter 7: Vitamin D: Molecular Biology and Gene Regulation in Singh AK and William GH. Textbook of Nephro-Endocrinology. Elsevier Inc, Burlington, MA, USA, 2009.

Dusso AS, Pavlopoilous T, Naumovich L et al. p21 (WAF1) and transforming growth factor alpha mediate dietary phosphate regulation of parathyroid cell growth. Kidney Int, 2001; 59: 855-865.

Echenique-Elizondo M, Amondarian JA, Vidaur F et al. Parathyroid subcutaneous pre-sternal transplantation after parathyroidectomy for renal hyperparathyroidism. Long term graft function. World J Surg, 2007; 31: 1403.

Eknoyan GA. History of the Parathyroid Glands. American Journal of Kidney Diseases 1995; 26(5): 801-807.

Ellika S, Patel S, Aho T et al. Preoperative Localization of Parathyroid Adenomas Using 4-Dimensional Computed Tomography: A Pictorial Essay. Canadian Association of Radiologists Journal 2013; 64(3): 258-268.

Ellis H. Hailey and Bishop's Notable Names in Medicine and Surgery. London: HK Lewis, 1983; 196-198.

Fancy T, Gallagher D, Hornig JD. Surgical Anatomy of the Thyroid and Parathyroid Glands. Otolaryngol Clin N Am, 2010; 43: 221-227.

Fawchett DW, Jensh RP. Concise Histology. Hoddler Arnold Publication: CRC Press, United States of America, 2002. Page 259-261.

Flament JB, Delattre JF, Pluot M. Arterial blood supply to the parathyroid glands: Implications for thyroid surgery. Anat Clin, 1982; 3:279.

Foley RN, Li S, Liu J et al. The fall and rise of parathyroidectomy in U.S. haemodialysis patients 1992-2002. J Am Soc Nephrol, 2005; 16:210.

Fuster D, Ybarra J, Ortin J et al. Role of preoperative imaging using 99mTc-MIBI and neck ultrasound in patients with secondary hyperparathyroidism who are candidates for subtotal parathyroidectomy. European Journal of Nuclear Medicine and Molecular Imaging, 2006; 33(4): 467-473.

Gagner M. Endoscopic subtotal parathyroidectomy in patients with primary hyperparathyroidism. Br J Surg, 1996; 83: 875.

Ghani A, Baxter P. Surgical Parathyroidectomy versus Cinacalcet Therapy: In the Management of Secondary Hyperparathyroidism. Otolaryngology-Head and Neck Surgery, 2012; 146 (2): 220-225.

Giddings CEB, Rimmer J, Weir N. History of parathyroid gland surgery: an historical case series. The Journal of Laryngology & Otology 2009; 123: 1075-1081.

Gilbert SJ, Weiner DE, Gipson DS et al. National Kidney Foundation's Primer on Kidney Diseases: Chapter 55- Bone Disorders in Chronic Kidney Disease Page 476-487. El Sevier Saunders, Philadelphia, PA, United States of America, 2014.

Gilmour JR, Grocers. The embryology of the parathyroid glands, the thymus and certain associated rudiments. The Journal of Pathology and Bacteriology, 1937; 45(3): 507-522.

Glasby MA, Huang CLH. Applied Physiology for Surgery and Critical Care. Elsevier- Health Sciences Division. London, UK, 1995. Page 452-454.

Gley ME. Comptes rendus des séances de la Société de biologie ed de ses filiales. 43, 551. 1891.

Gonzalez EA and Martin KJ. Chapter 69: Bone and Mineral Metabolism in Chronic Renal Failure in Johnson RJ and Feehally J (2000). Comprehensive Clinical Nephrology. Harcourt Publishers Limited, Barcelona, Spain, 2000.

Gray SW, Skandalakis JE, Akin JT Jr. Embryological considerations of thyroid surgery: Developmental anatomy of the thyroid, parathyroids and the recurrent laryngeal nerve. Am Surg. 1976; 42: 621-628.

Green J, Kleeman C. Role of bone in regulation of systemic acid-base balance [editorial review]. Kidney Int. 1991; 39: 9-36.

Greenson JK, Hornick JL, Longacre TA et al. Sternberg's Diagnostic Surgical Pathology. Chapter 13: Thyroid and Parathyroid, Page 572-583. Wolters Kluwe Health, USA, 2015.

Grevellec A, Tucker AS. The pharyngeal pouches and clefts: Development, evolution, structure and derivatives. Seminars in Cell and Developmental Biology, 2010; 21: 325-332.

Guerra R, Auyanet I, Fernandez EJ et al. Hypercalcemia secondary to persistent hyperparathyroidism in kidney transplant patients: analysis after a year with cinacalcet. J Nephrol, 2011; 24 (1): 78-82.

Gwiasda J, Kaltenborn A, Muller JA et al. Ultrasound based scores as predictors for nodular hyperplasia in patients with

secondary hyperparathyroidism: a prospective validation study. Langenbecks Arch Surg, 2017; DOI 10.1007/s00423-016-1546-5.

Ham A and Cormack. Histology Eighth Edition, Page 810-813. Lippincott, Philadelphia, United States of America, 1989.

Heath DA and Marx SJ. Clinical Endocrinology 2 Calcium Disorders; Liebross BA and Coburn JW Chapter 7- Renal osteodystrophy Page 151-188. Butterworth and Co (Publishers) Ltd. United Kingdom, 1982.

Heptinstall RH. Chapter 29: Calcium and the Kidney, Renal osteodystrophy and Stone Formation in Pathology of the Kidney. Little, Brown and Company, USA, 1983.

Herrera MF, Gamboa-Dominguez A Chapter 38: Parathyroid Embryology, Anatomy and Pathology. In: Textbook of Endocrine Surgery, 2nd edition. Elsevier, USA, 2005.

Hoang JK, Williams L, Gaillard F et al. Parathyroid 4D-CT: Multi-institutional International Survey of Use and Trends. Otolaryngol Head Neck Surg 2016; 155(6): 956-960.

Hoyes AD, Kershaw DR. Anatomy and development of the thyroid gland. Ear Nose Throat J, 1985; 64: 318-333.

Hu MC, Shi M, Zhang J et al. Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol, 2011; 22: 124.

Idem. Function parathyroidienne et function thyroidienne. Arch.Ital Biol 1900; 33: 154-156.

Ito F, Sippel R, Lederman J, Chen H. The utility of intraoperative bilateral internal jugular venous sampling with rapid parathyroid hormone testing. Ann Surg, 2007; 245: 959.

Ivarsson KM, Akaberi S, Isaksson E et al. The effect of parathyroidectomy on patient survival in secondary hyperparathyroidism. Nephrol Dial Transplant, 2015; 30: 2027-2033.

Jehle S, Hutler HN, Krapf P. Effect of potassium citrate on bone density, microarchitecture, and fracture risk in healthy older adults without osteoporosis: a randomized control trial. J Clin Endocrinol Metab, 2013; 98: 207-217.

Jowsey J, Reiss E, Canterbury JM. Long term effects of high phosphate intake on parathyroid hormone levels and bone metabolism. Acta Orthop Scand, 1974 45: 801-808.

Junquiera LC, Carniero J, Kelly RO. Basic Histology. Page 416-419. McGraw-Hill Education-Europe, Maidenhead, United States of America, 1998.

Kacsoh B. Endocrine Physiology, Page 157-158. Mc-Graw Hill/Appleton and Lange, United States of America, 2000.

Kacsoh B.Endocrine Physiology. McGraw Hill Education-Europe, London, UK, 2000. Page 150-175.

Kant KS, Glueck HI, Coots MC et al. Protein S deficiency and skin necrosis associated with continuous ambulatory peritoneal dialysis. Am J Kidney Dis, 1992; 19: 264-271.

Kasper DL, Fauci AS, Longo DL, Braunwald EB, Hauser SL, Jameson JL. Harrison's Principles of Internal Medicine. McGraw-Hill Companies, Inc, USA, 2005. Page 2249-2270.

Kawata R, Kotetsu L, Takamaki A et al. Ultrasonography for preoperative localization of enlarged parathyroid glands in secondary hyperparathyroidism. Auris Nasus Larynx, 2009; 36: 461-465.

Kidigo- Mineral Bone Disorder, 2015. Website: http://kdigo.org/home/mineral-bone-disorder/ Accessed on February 14, 2017.

Kim J, Jones BW, Zock C, Chen Z, Wang H, Goodman CS et al. Isolation and characterization of mammalian homologs of the Drosophila gene glial cells missing. Proc Natl Acad Sci USA, 1998; 95(21): 12364-12369.

Komaba H, Taniguchi M, Wada A et al. Parathyroidectomy and survival among Japanese haemodialysis patients with secondary hyperparathyroidism. Kidney Int, 2015; 88 (2) 350-359.

Komaba H, Kakuta T, Fukagawa M. Management of secondary hyperparathyroidism: how and why? Clin Exp Nephrol, 2017; DOI 10.1007/s10157-016-1369-2.

Konturek A, Barczynski M, Stopa M, Nowak W. Subtotal parathyroidectomy for secondary renal hyperparathyroidism: a 20 year surgical outcome study. Langenbecks Arch Surg, 2016; DOI 10.1007/s00423-016-1447-7.

Kraut JA, Shinaberger J, Singer FR, Sherrard DJ et al. Reduced parathyroid response to acute hypocalcaemia in dialysis osteomalacia. Clinical Research, 1981; 29: 102A.

Lafrance JP, Cardinal H, Leblanc M et al. Effect of cinacalcet availability and formulary listing on parathyroidectomy rate trends. BMC Nephrology, 2013; 14: 100.

Lawrence DAS. A histological comparison of adenomatous and hyperplastic parathyroid glands. Journal of Clinical Pathology, 1978; 31: 626-632.

Lazar ES and Stankus N. Cinacalcet-Induced Hungry Bone Syndrome. Seminars in Dialysis, 2007; 20: 83-85.

Leeson R, Leeson T, Papard, A. Textbook of Histology, Page 449-451. W.B Saunder, United States of America, 1985.

Levin A, Bakris GL, Molitch M et al. Prevalence of abnormal serum vitamin D, PTH, calcium and phosphorous in patients with chronic kidney disease: results of the study to evaluate early kidney disease. Kidney Int, 2007; 71: 31.

Li S, Chen YW, Peng Y, Foley RN, St Peter WL. Trends in parathyroidectomy rates in U.S. haemodialysis patients from 1992 to 2007. Am J Kidney Dis, 2011; 57(4): 602-611.

Liebross BA and Coburn A. Chapter 7: Renal osteodystrophy in Heath DA and Marx SJ, Clinical Endocrinology 2- calcium disorders. Butterworth and Co (Publishers), Cornwall, England, 1982.

Liu Z, Yu S, Manley. Gcm2 is required for the differentiation and survival of parathyroid precursor cells in the parathyroid/thymus primordia. Dev Biol. 2007; 305(1): 333-346.

Llach F. Secondary hyperparathyroidism in renal failure: the trade off hypothesis revisited. Am J Kidney Disease, 1995; 25: 663.

Lorenz K, Bartsch DK, Sancho JJ et al. Surgical management of secondary hyperparathyroidism in chronic kidney disease- a consensus report of the European Society of Endocrine Surgeons, 2015; 400: 907-927.

Lorenz K, Ukkat J, Sekulla C et al. Total parathyroidectomy without auto transplantation for renal hyperparathyroidism: experience with a qPTH-controlled protocol. World J Surg, 2006; 30 (5): 743-751.

Lumachi F, Basso SM. Pathophysiology and treatment of nonfamilial hyperparathyroidism. Recent Pat CNS Drug Discov, 2014; 9: 164.

Lumachi F, Ermani M, Basso S, Zucchetta P, Borsato N, Favia G. Localization of parathyroid tumours in the minimally invasive era: which technique should be chosen? Population based analysis of 253 patients undergoing parathyroidectomy and factors affecting parathyroid gland detection. Endor Relat Cancer, 2001; 8: 63-69.

Lundstroem AK, Trolle W, Soerensen CH et al. Preoperative localization of hyper functioning parathyroid glands with 4D-CT. Eur Arch Otorhinolaryngol. 2015; 273: 1253-1259.

MacCallum WG, Lambert RA, Vogel Km. The removal of calcium from the blood by dialysis in the study of tetany. J Exp Med, 1914; 20: 149-68.

Manley, N. Embryology of the Parathyroid Glands. Brandi, ML, Brown EM. Hypoparathyroidism. Page 11-18, Springer-Verlag, Italia, 2015.

Massry SG, Coburn JW, Lee DBN, Jowsey J and Kleeman CR. Skeletal resistance to parathyroid hormone in renal failure: a study of 105 human subjects. Annals of Internal Medicine, 1973; 78: 357-364.

McMinn RMH. Lasts Anatomy Regional and Applied Ninth Edition. Chapter 6, 432-433. London, United Kingdom 2010.

Messa P, Cafforio C, Alfieri C. Clinical impact of hypercalcaemia in kidney transplant. Int J Nephrol, 2011; 906832.

Meyers MO, Russell CP, Ollila DW, Yeh JJ, Kim HJ, Calvo BF. Postoperative hypocalcaemia after Parathyroidectomy for Renal Hyperparathyroidism in the Era of Cinacalcet. The American Surgeon, 2009; 75 (9): 843-847.

Michael J, Sircar S. Fundamentals of Medical Physiology. Thieme Medical Publishers, New York, USA, 2010. Page 497-499.

Milas M. Parathyroidectomy in end stage renal disease. In UpToDate, Wolters Kluwer, Netherlands, 2016.

Modarai B, Sawyer A, Ellis H. The glands of Owen. Journal of the Royal Society of Medicine 2004; 97: 494-495.

Moffett D, Moffett S, Schauf C. Human Physiology- Second Edition. Mosby-Year Book, Incorporated, USA, 1993. Page 577-579.

Mohebati A, Shaha AR. Anatomy of Thyroid and Parathyroid Glands and Neurovascular Relations. Clinical Anatomy 2012; 25: 19-31.

Moore KL, Dalley AF, Agur A. Clinically Oriented Anatomy, Chapter 8, 1020-1021. Baltimore, MD; United States of America, 2010.

Moore KL, Persaud TVN, Torchia MG. The Developing Human- Clinically Oriented Embryology 9th Edition. Saunders, Elsevier Inc, Philadelphia, PA, USA, 2013.

Moore KL, Persaud TVN. The Developing Human: Clinically Oriented Embryology (ed 6). Philadelphia, PA, Saunders, 1998.

Moralidis E. Radionuclide parathyroid imaging: a concise, updated review. Hell J Nucl Med, 2013; 16(2): 125-133.

Nobel Prize.org. The Nobel Prize in Physiology and Medicine 1977. Website:http://www.nobelprize.org/nobel_prizes/medicine/laureates/1977/yellow-facts,html. Last accessed 26 October, 2017.

Norman J, Lopez J, Politz D. Cinacalcet (Sensipar) Provides no Measurable Clinical Benefits for Patients with Primary Hyperparathyroidism and may

Accelerate Bone Loss with Prolonged Use. Ann Surg Oncol, 2012; 19: 1466-1471.

Nussbaum SR, Thompson AR, Hutcheson KA et al. Intraoperative measurement of parathyroid hormone in the surgical management of hyperparathyroidism. Surgery, 1998; 104: 1121-1127.

O'Rahilly R, Muller F. Human Embryology and Teratology (ed 2). New York, NY, Wiley-Liss, 1996.

Ohe MN, Santos RO, Kunii IS et al. Intraoperative PTH cut-off definition to predict successful parathyroidectomy in secondary and tertiary hyperparathyroidism. Braz J Otorhinolaryngol, 2013; 79: 494.

Organ CH. The History of Parathyroid Surgery, 1850-1996: The Excelsior Surgical Society 1998 Edward D Churchill Lecture. American College of Surgeons, 2000; 191 (3)Z: 284-299.

Pappenheimer AM, Wilens SL. Enlargement of the parathyroid glands in renal disease. Am J Pathol, 1935; 11: 73-91.

Parfrey PS, Chertow GM, Block GA et al. The clinical course of treated hyperparathyroidism among patients receiving haemodialysis and the effect of cinacalcet: the EVOLVE trial. Journal of clinical endocrinology Metabolism, 2013; 98: 4834.

Park JH, Kang SW, Jeong JJ et al. Surgical treatment of tertiary hyperparathyroidism after renal transplantation: A 31 year experience in a single institution. Endocrine Journal, 2011; 58: 827-833.

Pathology Outlines: Parathyroid Gland, Hyperparathyroidism, Parathyroid gland hyperplasia, 2013. Website: www.pathologyoutlines.com/topic/parathyroidpthhyper.html. Last accessed 24 October, 2017.

Pierides AM, Skillen AW, Ellis H. Serum alkaline phosphatase in azotemic and haemodialysis osteodystrophy: a study of isoenzyme patterns, their correlation with bone histology, and their changes in response to treatment with 1aOHD3 and 1,25(OH)2D3. Journal of Laboratory Clinical Medicine, 1979; 93: 899-909.

Policeni BA, Smoker WRK, Reede D. Anatomy and Embryology of the Thyroid and Parathyroid Glands. Semin Ultrasound CT and MRI, 2012; 33: 104-114.

Policeni BA, Smoker WRK, Reede DL. Anatomy and Embryology of the Thyroid and Parathyroid Glands. Semin Ultrasound CT and MRI, 2012; 33: 104-114.

Poon G. Cinacalcet hydrochloride (Sensipar). Proc (*Bayl Univ BMed Cent), 2005; 18(2): 181-18.

Prinz RA, Lonchyna V, Carnaille B et al. Thoracoscopic excision of enlarged mediastinal parathyroid glands. Surgery, 1994; 116: 999-1004.

Quarles LD, Berkoben M. Management of secondary hyperparathyroidism and mineral metabolism abnormalities in dialysis patients. In UpToDate, Wolters Kluwer, USA, 2015.

Qunibi WY, Henrich W. Overview of chronic kidney disease-mineral bone disease (CKD-MBD). Up To Date, Wolters Kluwer, Netherlands, 2015.

Raff H, Levitzky M. Medical Physiology- A Systems Approach. McGraw Hill Professional, USA, 2011. Page 643-651.

Rafferty K, Davis KM, Heaney RP. Potassium intake and the calcium economy. J Am Coll Nutr 2005; 24: 99-106.

Randolph G. Surgery of the thyroid and parathyroid glands. Philadelphia: WB Saunders, USA, 2003.

Richards ML, Wang TS, Sosa JA. Surgical anatomy of the parathyroid glands. Up To Date, Wolters Kluwer, Netherlands 2015.

Rodgers SE, Hunter GJ, Hamberg LM et al. Improved preoperative planning for directed parathyroidectomy with 4-dimensional computed tomography. Surgery 2006; 140(6): 932-40.

Rodriguez M, Felsenfeld AJ, Llach F. Calcaemic response to parathyroid hormone in renal failure: role of calcitriol and the effect of parathyroidectomy. Kidney Int, 1991; 40: 1063.

Rogers-Stevane J, Kauffman GL Jr. A Historical Perspective on Surgery of the Thyroid and Parathyroid Glands. Otolaryngologic Clinics of North America, 2008; 41: 1059-1067.

Roher HD, Schulte KM History of Thyroid and Parathyroid Surgery. In Surgery of the Thyroid and Parathyroid Glands, Chapter 1, Page 1-12, Springer 2007, Heidelberg, Germany.

Rolleston HD. The Endocrine Organs in Health and Disease, with an Historical Review. London, UK, Oxford University Press, 1936.

Ross MH, Pawlina W. Histology- A Text and Atlas Sixth Edition. Page 705-706. Lippincott Williams and Wilkins, United States of America, 2006.

Rostaing L, Moreau-Gaudry X, Baron E et al. Changes in blood pressure and renal function following subtotal parathyroidectomy in renal transplant patients presenting with persistent hypercalcaemic hyperparathyroidism. Clin Nephrol, 1997; 47: 248.

Roth SI. The Parathyroid Gland, Figure 57-38 In: Silverberg SA, DeLellis Principles and practice of surgical pathology and cytopathology 3rd edition. Churchill Livingstone, USA, 1997.

Rothmund M, Wagner PK, Schark C. Subtotal parathyroidectomy versus total parathyroidectomy and auto transplantation in secondary hyperparathyroidism: a randomized trial. World J Surg, 1991; 15: 745.

Rothmund M, Wagner PK, Schark C. Subtotal parathyroidectomy versus total parathyroidectomy and auto transplantation in secondary hyperparathyroidism: a randomized trial. World J Surg, 1991; 15 (6): 745-750.

Rudser KD, de Boer IH, Dooley A. Fracture risk after parathyroidectomy among chronic haemodialysis patients. J Am Soc Nephrol, 2007; 18: 2401.

Rutherford WE, Bordier P, Marie P et al. Phosphate control and 25-hydroxycholecalciferol administration in preventing experimental renal osteodystrophy in the dog. Journal of Clinical Investigation, 1977; 60: 332-341.

Safford SD, Skinner MA. Thyroid and parathyroid disease in children. Semin Pediatr Surg. 2006; 15: 85-91.

Sandstrőm I. Om en ny kőrtel hos meniskan och atskilliga daggdjur. Upsala Lakareforenings Főrhandlingar band XV: 441-471.

Schlosser K, Bartsch DK, Diener MK et al. Total parathyroidectomy with routine thymectomy and auto transplantation versus total parathyroidectomy alone for secondary hyperparathyroidism: results of a nonconfirmatory multicentre prospective randomized controlled pilot trial. Ann Surg, 2016; 264: 745.

Schneider R, Bartsch DK. Role of surgery in the treatment of renal secondary hyperparathyroidism. British Journal of Surgery, 2014; 102: 289-290.

Sebastian A, Harris ST, Ottaway JH et al. Improved mineral balance and

skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med 1994; 330(25): 1776-81.

Seehofer D, Steinmuller T, Rayes N et al. Parathyroid hormone venous sampling before re-operative surgery in renal hyperparathyroidism: comparison with non-invasive localisation procedures and review of the literature. Arch Surg, 2004; 139(12): 1331-1338.

Shimada T, Kakitani M, Yamazaki Y et al. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest, 2004; 113: 561-568.

Slatopolsky E, Finch J, Denda M et al. Phosphorous restriction prevents parathyroid gland growth- high phosphorous directly stimulates PTH secretion in vitro. J Clin Invest, 1996; 97: 2534-2540.

Slatopolsky E, Robson AM, Elkan I, Brinker NS. Control of phosphate excretion in uraemic man. J Clini Invest, 1968; 47: 1865.

Slatopolsky E, Caglar S, Pennell JP et al. On the pathogenesis of hyperparathyroidism in chronic experimental renal insufficiency in the dog. Journal of Clinical Investigation, 1971; 50: 492-499.

Som PM, Curtin HD. Head and Neck Imaging (ed 4). St Louis, MO, Mosby, 2003.

Tang B, Moreno-Reyes R, Blocklet D et al. Accurate pre-operative localization of pathological parathyroid glands using 11C-methionine PET/CT. Contrast Media Mol. Imaging, 2008; 3: 157-163.

The Doctors' Doctor: Parathyroid Hyperplasia, 2005. Website: www.thedoctorsdoctor.com/diseases/parathyroid_hyperplasia,htm. Last accessed 25 October, 2017.

Thompson NW. The history of hyperparathyroidism. Acta chir Scand 1990; 156: 5-21.

Tibblin SA, Bondeson AG, Ljungberg O. Unilateral parathyroidectomy in hyperparathyroidism due to single adenoma. Ann Surg, 1982; 195: 245-252.

Toft A, Campbell I, Seth J. Diagnosis and Management of Endocrine Diseases: Chapter 18- Hyperparathyroidism and hypoparathyroidism Page 354-378. Blackwell Scientific Publications. London, Great Britain, 1981.

Toneto MG, Prill S, Debon LM et al. The history of parathyroid surgery. Rev Col Bras Cir, 2016; 43(3): 214-222.

Tsuruta Y, Okano K, Kikuchi K, Tsuruta Y, Akiba T, Nitta K. Effects of cinacalcet on bone mineral density and bone markers in haemodialysis patients with secondary hyperparathyroidism. Clin Exp Nephrol, 2013; 17: 120-126.

Tylavsky FA, Spence LA, Harkness L. The importance of calcium, potassium, and acid base homeostasis in bone health and osteoporosis prevention. Journal of Nutrition. 138(1): 164S-165S.

Tzanakis I, Alifieris E, Kagia S et al. Does parathyroidectomy affect residual diuresis in haemodialysis patients? Nephron, 2000; 86: 402.

Urakawa I, Yamazaki Y, Shimada T et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature, 2006; 444: 770.

Urena P, Jacobson SH, Vevloet M et al. Cinacalcet and achievement of the NKF/K-DOQI recommended target values for bone and mineral metabolism in real-world clinical practice- the ECHO observational study. Nephrol Dial Transplant, 2009; 24: 2852-2859.

Vassale G, Generali F. On the effects of extirpation of the parathyroid glands. Rif Patol nerv Ment, 1896; 1: 249-252.

Vassale G, Generali F. On the effects of extirpation of the parathyroid glands. The Alienist and Neurologist, 1897; 18: 57-61.

Vulpio C, Bossola M, De Gaetano A et al. Usefulness of the combination of ultrasonography and 99MTC-sestamibi scintigraphy in the preoperative evaluation of uraemic secondary hyperparathyroidism. Head and Neck, 2010; DOI 10.1002/hed: 1226-1235.

Wakamatsu H, Noguchi S, Yamashita H et al. Parathyroid scintigraphy with 99m Tc-MIBI and 123I subtraction: a comparison with magnetic resonance imaging and ultrasonography. Nucl Med Commun, 2003; 24: 755.

Wang AY, Wang M, Joo J et al. Cardiac valve calcification as an important predictor for all-cause mortality and cardiovascular mortality in long term peritoneal dialysis patients: a prospective study. J Am Soc Nephrol, 2003; 14: 159-168.

Wang C. The anatomic basis of parathyroid surgery. Ann Surg, 1976;183: 271-275.

Weaver CM. Potassium and Health. Adv Nutu 2013; 4(3): 368S-377S

Wiese RJ, Uhland-Smith A, Ross TK et al. Upregulation of the vitamin D receptor in response to 1, 25-dihydroxyvitamin D3 results from ligand-induced stabilization. J Biol Chem, 1992; 267: 20082-20086.

Wirowski D, Goretzki PE, Schwarz K, Lammers BJ. Cinacalcet effects on the perioperative course of patients with secondary hyperparathyroidism. Langenbecks Arch Surg, 2013; 398: 131-138.

Xu D, Yin Y, Hou L, Dai W. Surgical management of secondary hyperparathyroidism: how to effectively reduce recurrence at the time of primary surgery. J Endocrinol Invest, 2016; 39: 509-514.

Yarlagadda SG, Nickolas TN, Quarles LD. Persistent hyperparathyroidism after renal transplantation. In UpToDate, Wolters, Kluwer, Netherlands, 2017.

Yip L, Silverberg SJ, Fuleihan GE. Preoperative localization for parathyroid surgery in patients with primary hyperparathyroidism. In UpToDate, Wolters Kluwer, Netherlands, 2015.

Yuan LL, Kan Y, Ma DQ et al. Combined application of ultrasound and SPECT/CT has incremental value in detecting parathyroid tissue in SHPT patients. Diagnostic and Interventional Imaging, 2016; 97(2): 219–225.

Yuen NH, Ananthakrishnan S, Campbell MJ. Hyperparathyroidism of renal disease. Perm J 2016; 20: 76-83.

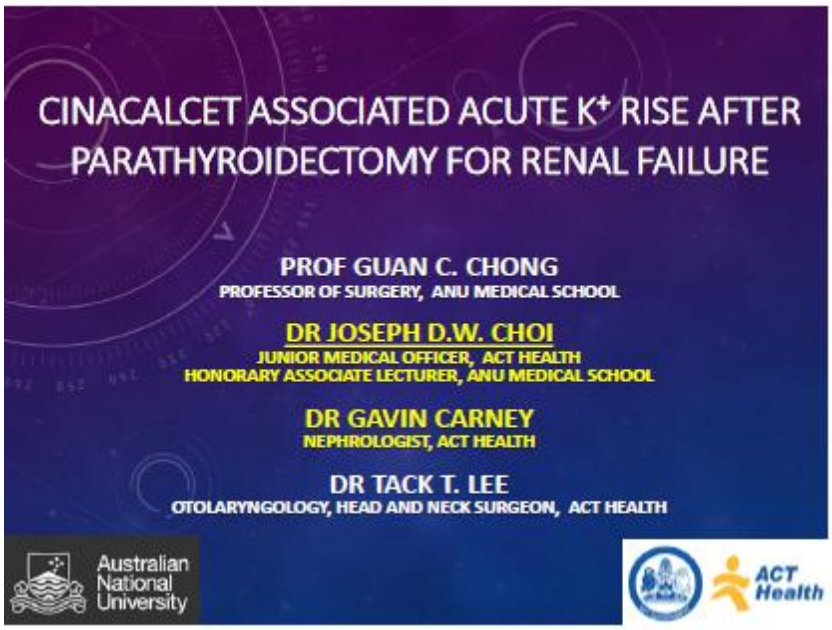
Zahoor T, Bajaj Y, Roberts S, England J. Intraoperative Hyperkalaemia: Death in Renal Parathyroidectomy. Otolaryngol Head Neck Surg, 2012; 147(2): P162.

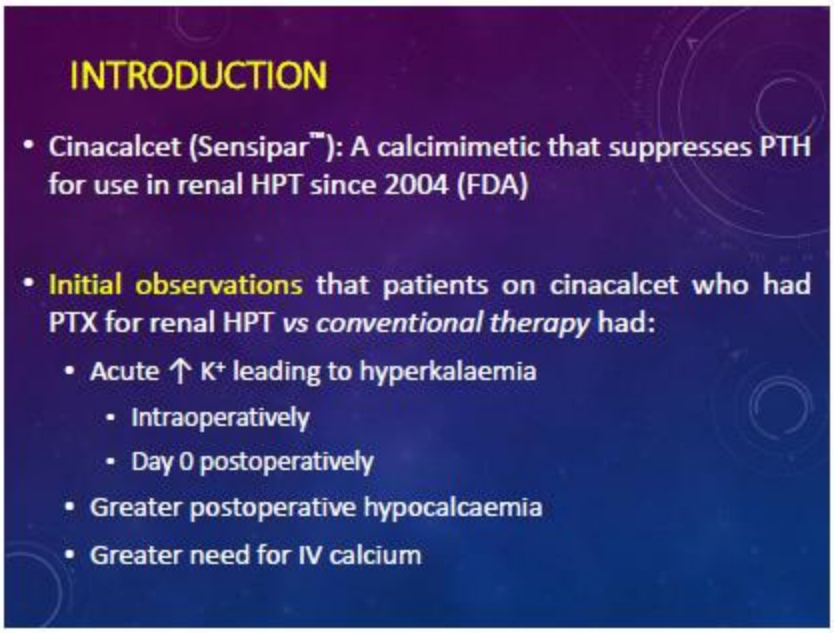
Zapanta PE, Meyers A. Embryology of the Thyroid and Parathyroids. Medscape 2016. WebMD. Website: https://emedicine.medscape.com/article/845125-overview. Last accessed 24 October, 2017.

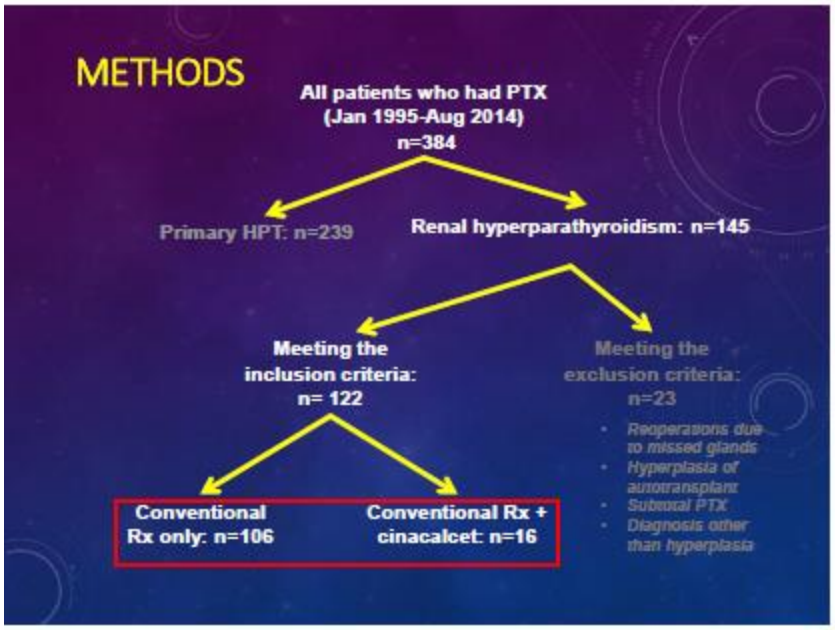
Zitt E, Rix M, Torres PU et al. Effectiveness of cinacalcet in patients with recurrent/persistent secondary hyperparathyroidism following parathyroidectomy: results of the ECHO study. Nephrol Dial Transplant, 2011; 26: 1956-1961.

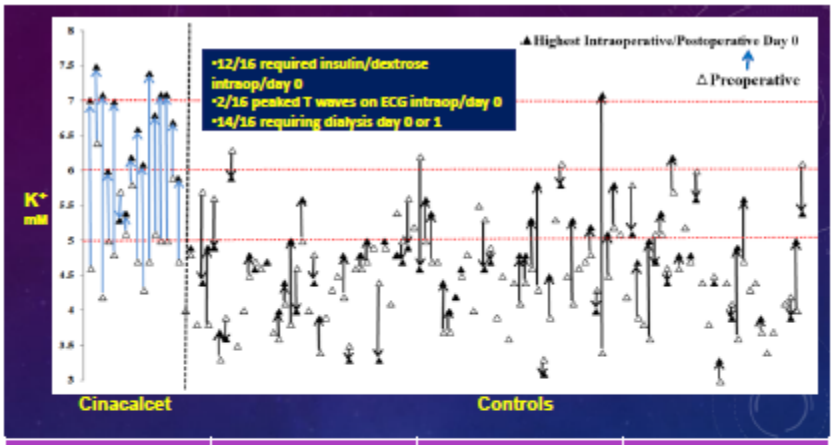
PART VI: APPENDIX

(i) **Power-point Slides for Oral Presentation Acceptance at General Surgeons Australia Annual Scientific Meeting, 2014 (GSA ASM 2014)**



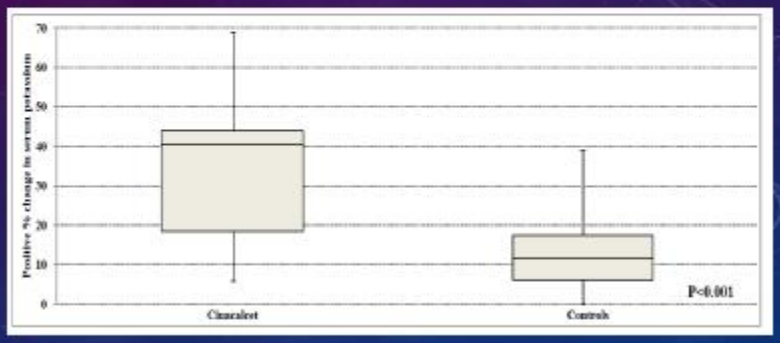




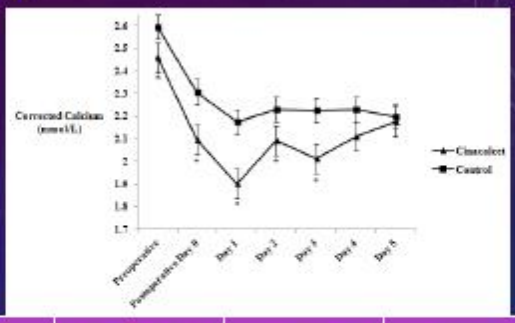


Potassium	Cinacalcet	Controls	P Value
Intra/post op D0 K>6	81.25% (13/16)	2.99% (2/67)	<0.001
Intra/post op D0 K>7	43.75% (7/16)	1.49% (1/67)	<0.001
Δ >+1mM	75.00% (12/16)	11.94% (8/67)	<0.001
Mean Δ pre→postop	+1.51 ± 0.93 mM	+0.19 + 0.77 mM	<0.001

POSITIVE % CHANGE OF POTASSIUM FROM PREOP VALUE



RESULTS



Characteristic	Total (n=122)	Cinacalcet (n=16)	Control (n=106)	P Value
IV calcium postoperatively	61	15 (93.75%)	46 (43.40%)	<0.001
Postoperative stay (days)	5.83 ± 2.75	7.40 ± 3.00	5.59 ± 2.65	<0.05

DISCUSSION

Intra-operative hyperkalemia: a serious but under recognised complication of renal parathyroidectomy – a prospective study: how we do it

Bajaj, Y., Roberts, S., Simon, D., Snowden, C., Gianopoulos, I. & England, R.J.

Hull Royal Infirmary, Hull, UK

Accepted for publication 6 December 2010

Clinical Otolaryngology
Volume 36, Issue 1, pages 69–72, February 2011

- N=29 patients who had PTX for secondary HPT
- N=16 (55%) had intraoperative hyperkalaemia
- N=1 reported intraoperative K^+=7.8mM, peaked *t wave* and death after two glands removed
- No comment on cinacalcet or calcium status

DISCUSSION

Postoperative Hypocalcemia after Parathyroidectomy for Renal Hyperparathyroidism in the Era of Cinacalcet

MICHAEL O. MEYERS, M.D., CHRISTINA E. RUSSELL, B.A., DAVID W. OLLILA, M.D., JEN JEN YEH, M.D., HONG JIN KIM, M.D., BENJAMIN F. CALVO, M.D.

From the Department of Surgery and The Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

THE AMERICAN SURGEON September 2009

- N=63 (controls), N=14 (cinacalcet)
- More Px on cinacalcet required IV calcium postoperatively (62%) vs controls (41%)
  - *Our data: 93.75% cinacalcet vs 43.40% controls*
- Cinacalcet led to a lower postoperative nadir Ca vs. controls suggesting more aggressive postop course
- No comment on hyperkalaemia

PROTOCOL- PERIOPERATIVE MANAGEMENT

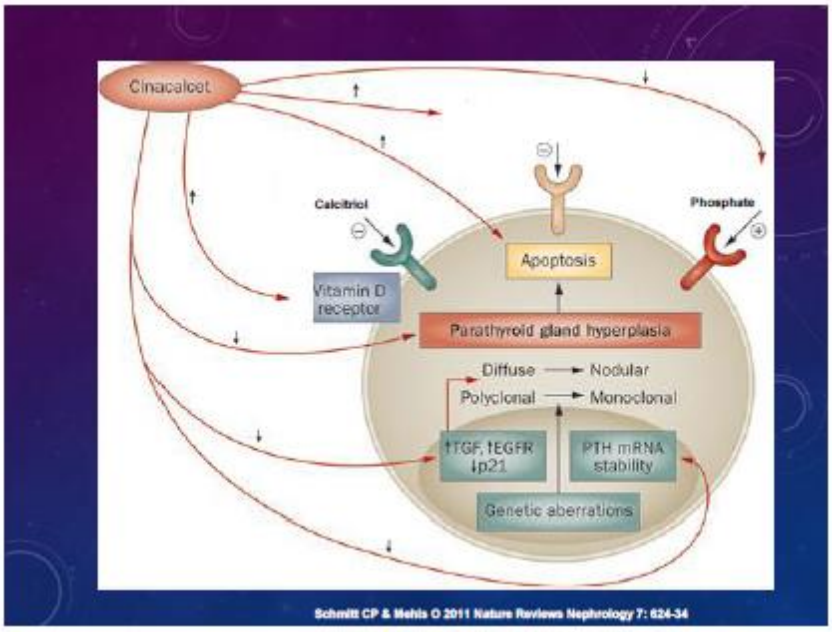
1. Dialyse patients 24 hours before operation
2. Check serum potassium, calcium, magnesium, phosphate and alkaline phosphatase prior to operation
3. On the day of operation, place a central line or peripherally inserted central catheter, and an arterial line before surgery
4. Continuously monitor ECG for tall peaked *t*-wave, shortened *QT* interval, lengthening of *PR* interval and *QRS* complexes intraoperatively
5. Analyse arterial blood for potassium and ionised calcium hourly and to time with the removal of first and fourth parathyroid glands
6. (a) Administer 10 units of IV insulin in 50% dextrose in 50ml if serum potassium >6.0mmol/L

   (b) Administer additional bolus dose of IV 10% calcium gluconate in 10ml given if (a) fails to reduce serum potassium level
7. Consider admitting patient to high dependency unit after operation
8. Monitor calcium, magnesium, phosphate and potassium every two hours in the postoperative day 0 period, and four hourly in the postoperative day 1 period
9. Administer oral calcium carbonate and calcitriol every three to six hourly and give bolus dose of IV calcium if corrected calcium < 1.90 mmol/L
10. Consider dialysis in the postoperative day 0 period if serum potassium is not well controlled by 10 units of IV insulin in 50% dextrose in 50ml and IV calcium.

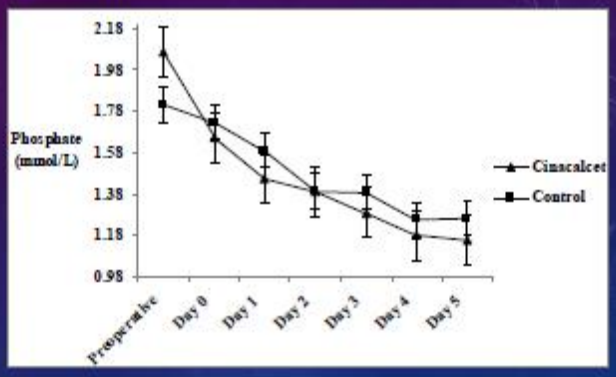
CONCLUSION

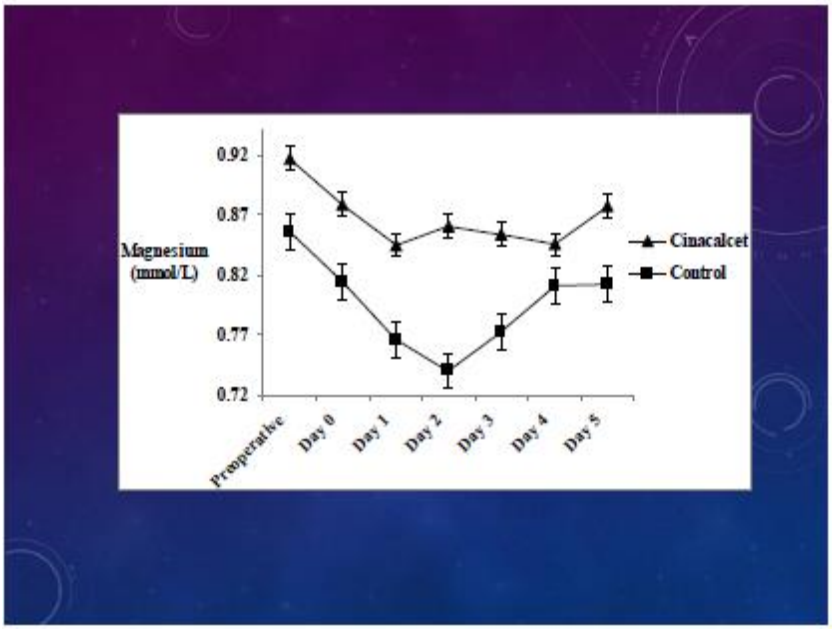
- Mechanism unknown, but proposed that:
  - *Intracellular K^+ mobilises out into the extracellular space to maintain electrical neutrality when Ca^{2+} is substantially reduced after PTX*
- Findings lead to alteration in protocol at our institution

APPENDIX


Cinacalcet
Calcitriol
Vitamin D receptor
Phosphate
Apoptosis
Parathyroid gland hyperplasia
Diffuse → Nodular
Polyclonal → Monoclonal
↑TGF, ↑EGFR ↓p21
PTH mRNA stability
Genetic aberrations
Schmitt CP & Mehls O 2011 Nature Reviews Nephrology 7: 624-34

- Secondary and tertiary HPT are frequently observed in Px with ESRF
  - ↑ $PO4^{3-}$, ↓Ca^{2+}, ↓calcitriol leads to ↑PTH and diffuse parathyroid hyperplasia
  - Progression leads to nodular hyperplasia, ↓ vitamin D receptors and ↓ CaS receptor
- Current management includes
  - Medical
    - PO4 binders/calcitriol/Ca/calcimimetics
  - Surgical
    - PTX
    - RTX




Magnesium (mmol/L)
0.92
0.87
0.82
0.77
0.72
Preoperative
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Cinacalcet
Control

(ii) **Publication in Clinical Otolaryngology:** Chong GC, **Choi JDW**, Lee TT. Carney G. Intraoperative and postoperative hyperkalaemia after total parathyroidectomy following exposure to cinacalcet in sixteen patients for renal hyperparathyroidism. *Clinical Otolaryngology 2017;* doi: 10.1111/coa.12880. [Epub ahead of print].